EFFECT OF SORGHUM TYPE AND PROCESSING ON THE ANTIOXIDANT PROPERTIES OF SORGHUM [*Sorghum bicolor* (L.) Moench] BASED FOODS

A Dissertation

by

NOMUSA RHODA NGWENYA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Food Science and Technology

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ABSTRACT

Effect of Sorghum Type and Processing on the Antioxidant Properties of Sorghum [*sorghum bicolor* (L. Moench)] Based Foods. (May 2007)

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Antioxidant properties of sorghum are related to sorghum type and method of processing into foods. Tannin and non-tannin sorghums and their products were evaluated for total phenols, tannins and antioxidant activity. Total phenols were determined using the Folin Ciocalteu method, and tannins were determined by the vanillin-HCl method. Antioxidant activity was evaluated using the ABTS (2,2′-azinobis(3-ethyl-benzothiazoline-6-sulphonic acid) and DPPH (2,2′-diphenyl-I-picrylhydrazyl) assays. Tannin sorghums and their products had higher total phenols, tannins and antioxidant activity than non-tannin sorghum grain and products. Fermentation, extrusion cooking and porridge making reduced measurable phenols, tannins and *in vitro* antioxidant activity. Reduction was probably due to phenols binding to the food components, thus reducing their solubility in the extracting solvents; 1% HCl in methanol and 70% aqueous acetone.

The procyanidin profile obtained using normal phase HPLC and fluorescent detection showed that extrusion cooking and porridge making lowered extractability of polymers (DP>8), while that of oligomers (DP 2-8) and monomers in porridges was not significantly changed. This indicated increased interactions of procyanidin polymers with the food matrix, especially with protein. Pepsin treatment of sorghum extrudates and porridges significantly improved the antioxidant activity and recovery. The highest antioxidant activity was in the supernatants of pepsin hydrolysates. Amylase treatment alone did not significantly affect phenol content and antioxidants,

except in bread containing non-tannin white sorghum bran, where there was a slight increase in phenols. The combination of pepsin followed by amylase treatment of porridges and extrudates had effects similar to those of pepsin alone.

Improved extractability of antioxidants on pepsin treatment was due to either the release of phenolic antioxidants or protein hydrolysates high in aromatic amino acid residues such as tyrosine, also known for their antioxidant activity. In either situation the improved antioxidant activity could mean that once food is digested it can potentially protect the gastrointestinal tract against oxidative stress generated from the diet and that produced by food interactions during digestion.

There is scope to explore other biological methods like use of other proteases to improve antioxidant recovery. Further work would thus determine contribution of phenolic compounds to improved antioxidant activity, and also identify the phenolics.

DEDICATION

To my children Imani and Mandisa

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TABLE OF CONTENTS

Page

CHAPTER Page

CHAPTER Page

LIST OF TABLES

TABLE Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

FIGURE Page

CHAPTER I

INTRODUCTION

The role of dietary antioxidants in preventing the development of several human diseases is well documented. Antioxidants have the ability to scavenge free radicals, and thus may protect the body from oxidative and free radical damage which are implicated in the development of some cancers, degenerative diseases such as atherosclerosis, coronary heart disease and the aging process (Namiki 1990, Chung et al 1998). There have been suggestions that antioxidants could be of potential benefit in individuals infected by the human immunodeficiency virus (HIV) ([1]Liang et al 1998, Sepulveda and Watson 2002, Gil et al 2003).

There is increasing evidence that some phenolic compounds in the diet possess the ideal structure for free radical scavenging activities and some have been found to be more effective *in vitro* on molar basis, than vitamin E (Rice-Evans et al 1997). Sorghum [*Sorghum bicolor* (L.) Moench], like all other plants, contains phenolic compounds and these are divided into three major categories: phenolic acids, flavonoids and tannins (Serna-Saldivar and Rooney 1995). It is important to note that tannins are only found in sorghum varieties with a pigmented testa, those that posses the B_1B_2, genes or Type II and III sorghums (Waniska and Rooney 2000). The sorghum tannins are condensed tannins. Until recently, tannins have been considered strictly as anti-nutrients on account of their protein binding property, which reduces protein digestibility (Duodu 2000). However there is increasing evidence showing that tannins are powerful antioxidants (Hagerman et al 1998), and even when complexed to proteins, tannins retain some of their antioxidant activity (Riedl and Hagerman 2001).

Sorghum is a drought resistant crop and plays an important part in the diet of millions of people in semi-arid regions of sub-Saharan Africa, Asia, Central America and the Middle East (Doggett 1988, Kent and Evers 1994). Sorghum is ranked the fifth

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This dissertation follows the style and format of Cereal Chemistry.

most important cereal after wheat, rice, maize and barley and its world production is 59 million tons (FAOSTAT data. 2005). Sorghum varieties that have high phenolic content, such as anthocyanins and procyanidins; could be useful as functional foods and offer potential health benefits in terms of providing antioxidants (Awika and Rooney 2004, Dykes and Rooney 2006). Sorghum, as a gluten-free cereal could also be an important ingredient in gluten free cereal products. In 2004, gluten intolerance or celiac disease was estimated to affect one in every 133 Americans (Marcus 2006).

In order to explore the possible health benefits of sorghum in the diet there is need to investigate the effect of processing on antioxidant activity of sorghum-based foods. There is limited information on the effect of processing on the antioxidant properties of a wide variety of foods. Some data exists on enhanced antioxidant activity in bread and cookies with added tannin sorghum bran (Awika et al 2003a). Sorghum bran high in flavonoids and tannins was found to be good sources of antioxidants.

Different food products are prepared from sorghum; for example whole or decorticated the grain can be milled to produce meal or grits used for making porridges. The porridge can be either plain or fermented, for example the fermented porridge *Ting* from Southern Africa (Taylor and Taylor 2002). The sorghum grain can also be germinated during the preparation of malt products, which include porridges and some alcoholic and non-alcoholic beverages, like *Mahewu*, which is produced in Zimbabwe (Bvochora et al 1999). Technologies such as extrusion cooking are being explored in the production of instant or ready-to-eat sorghum food products such as breakfast cereals. It is hoped that use of these new technologies would increase industrial utilization of sorghum.

The objectives of the research were:

- To characterize non-tannin and tannin sorghum in terms of determination of phenol content, composition and antioxidant activity. The non-tannin sorghums (Type I) were the white tan plant Macia and a red sorghum, NK 283. The tannin (Type III) sorghums were Red Swazi, an open pollinated variety, NS 5511 and Framida.

- Determine the effect of primary processing procedures including decortication and milling, fermentation and extrusion on phenol content, composition and antioxidant activity of the different types of sorghum.
- Determine antioxidant activity, phenol content and composition of traditional sorghum products such as porridges and extruded products.
- Separation and quantification of procyanidins (condensed tannins) using normal-phase HPLC
- Determine effect of pepsin and amylase hydrolysis on extractable phenols and antioxidant activity of processed samples.

CHAPTER II

LITERATURE REVIEW

SORGHUM PHENOLIC COMPOUNDS, ANTIOXIDANT ACTIVITY AND PROCESSING

Structure of sorghum grain

The sorghum grain or kernel is considered to be a naked caryopsis, although some African types retain their glumes (Waniska and Rooney 2000). The grain's principal anatomical components are the pericarp (outer layer), testa or seed coat, the endosperm tissue and the germ (embryo and scutellum) (Serna-Saldivar and Rooney 1995) (Fig. 1). The proportionally large germ results in high oil content in the kernel, and this may lead to rancidity in the flour, thus the need to separate the germ from the kernel during milling (Taylor and Dewar 2001).

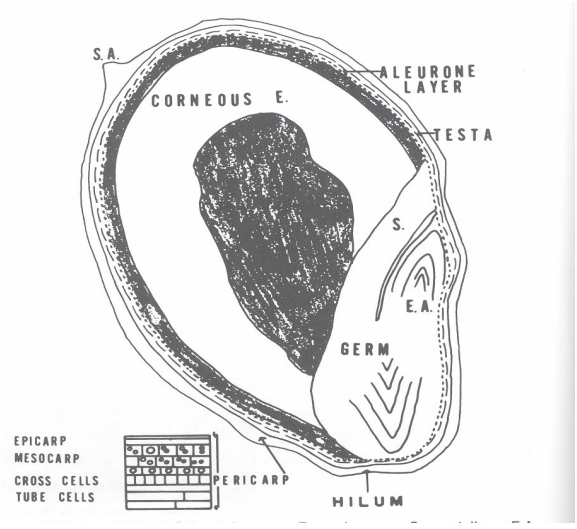

Fig. 1. Structure of the sorghum grain showing the pericarp, endosperm (aleurone layer, corneous and floury), and germ [scutellum (S) and embryonic axis (EA)].
(Waniska and Rooney 2000).

The pericarp

The pericarp of sorghum grain originates from the ovary and is divided into three histological tissues, the epicarp, mesocarp, and endocarp. The epicarp is the outermost layer and is generally covered with a thin layer of wax. The epicarp is two or three cell layers thick, the cells are rectangular in shape and may contain pigments. The mesocarp in sorghum is different from that of most cereals, in that it contains starch granules (Waniska and Rooney 2000). The endocarp is composed of cross and tube cells.

The color of the sorghum grain pericarp appears to be due to a combination of primarily anthocyanin and anthocyanidin pigments and other flavonoid compounds (Hahn et al 1984). The pigmentation of the pericarp is determined by the R and Y genes (Waniska and Rooney 2000). The pericarp is white or colorless when Y is homozygous recessive (rr yy or R- yy), lemon-yellow when Y is dominant and R is recessive (rr Y-) and red when both R and Y are dominant (R- Y-) (Hahn et al 1984). The Y gene is thought to be the basic gene for the synthesis of the flavonoid skeleton from phenolic acids, while the R gene appears to control the reduction of the flavonone to its corresponding flavan (Hahn et al 1984). The intensifier gene (I) affects the intensity of pericarp color and this is most readily apparent when the pericarp is red (R- Y-).

The testa

The seed coat (testa layer) may be highly pigmented, a characteristic that is genetically controlled (Waniska and Rooney 2000). In tannin sorghum, the testa is pigmented and this feature is controlled by the complementary B_1 and B_2 genes (Hahn et al 1984). For a pigmented testa to be present both must be dominant (B_1- B_2-). If one set or both is homozygous recessive (b_1b_1 B_2-, B_1- b_2b_2 or b_1b_1 b_2b_2), the pigmented testa is absent. The dominant B_1 and B_2 genes appear to control the polymerization of flavans (anthocyanidins) to the flavan-3-ol polymers (tannins) (Hahn et al 1984).

The endosperm

The endosperm tissue is composed of the aleurone layer, peripheral, corneous and floury areas. The aleurone is the outer layer of the endosperm and consists of a single layer of rectangular cells adjacent to the testa or tube cells. The cells possess a

thick cell wall, large amounts of proteins (aleurone grains, enzymes), ash (phytin bodies) and oil (spherosomes). The peripheral starchy endosperm is composed of several layers of dense cells containing more protein bodies and smaller starch granules. The corneous and floury endosperm cells are composed of starch granules, a protein matrix, protein bodies and cell walls rich in glucuronoarabinoxylans. The starch granules and protein bodies are embedded in the continuous protein matrix in the peripheral and corneous areas.

The germ

The germ is diploid due to the sexual union of one male and one female gamete. It consists of two major parts, the embryonic axis and scutellum. The embryonic axis contains the new plant and is divided into a radicle (new root) and plumule (shoot). The scutellum is the single cotyledon and contains reserve nutrients such as moderate amounts of oil, protein, enzymes and minerals.

The color of sorghum grain

The appearance of sorghum grain is affected by the color and thickness of the pericarp, the presence of a pigmented testa and the color of the endosperm (Hahn and Rooney 1986). If a pigmented testa is present, the color of the pericarp is brown when a dominant spreader gene, S is present. The intensity of the brown color of the pericarp depends on the genetic color of the pericarp, whether it is white, lemon-yellow or red. The spreader gene controls the presence of pigments and possibly tannins in the epicarp. When S is dominant, more phenols and tannins are in the pericarp and testa layers, than when it is recessive. This has led to the separation of brown sorghums into type II sorghums, those with a pigmented testa and recessive spreader gene (B_1- B_2- ss); type III sorghums have a pigmented testa and dominant spreader gene (B_1- B_2- S-) Type III sorghums have the highest level of tannins (Hahn and Rooney 1986). Type I sorghums do not have a pigmented testa and do not contain tannins.

The Z-gene controls pericarp thickness. Sorghums with homozygous recessive (zz) genes have a thick pericarp. A thick pericarp contains small starch granules, which

result in a chalky appearance that masks the color of the testa and endosperm (Serna-Saldivar and Rooney 1995).

Sorghum phenolic compounds

All sorghums contain phenolic compounds and these are divided into three major categories: phenolic acids, flavonoids and tannins (Serna-Saldivar and Rooney 1995). Free phenols are generally rare in plants, and a few that have been reported include catechol, orcinol phloroglucinol and pyrogallol (Harborne 1991). Free phenols are undesirable in food because they are carcinogenic, hepatotoxic and goitrogenic (Dicko et al 2006)

The term phenolic compound embraces a wide range of plant substances that possess in common, an aromatic ring bearing one or more hydroxyl substituents. Phenolic units are occasionally encountered in proteins, alkaloids and among terpenoids (Harborne 1991). Phenolic substances most frequently occur combined with sugar, as glycosides and they therefore tend to be water-soluble (Harborne 1991).

The biosynthesis of phenolic compounds in plants is initiated by the shikimic pathway, which continues with the production of phenylalanine. Phenylalanine and tyrosine are subsequently deaminated by the enzyme phenylalanine lyase into cinnamate derivatives (Dicko et al 2006) (Fig. 2 A).

Phenolic acids

Phenolic acids are derivatives of benzoic or cinnamic acids (Fig. 2) where the hydroxyl and methoxy groups are substituted at various places on the aromatic ring (Hahn et al 1984). The phenolic acids identified in sorghum include gallic, protocatechuic, vanillic, ferulic, caffeic, cinnamic, p-coumaric and p-hydroxybenzoic acids (Hahn et al 1983, Beta et al 1999). In cereal grains the phenolic acids exist as free acids, soluble and insoluble esters and are concentrated in the outer layers of the kernel (pericarp, testa and aleurone) (Hahn et al 1984). The only phenols found in the endosperm are the insoluble, tightly bound ones which appear to be associated with the cell walls of the grain. The major bound phenolic acid in sorghum is ferulic acid (3-

methoxy-4-hydroxy-cinnamic acid) which is associated with cell walls (Hahn et al 1984).

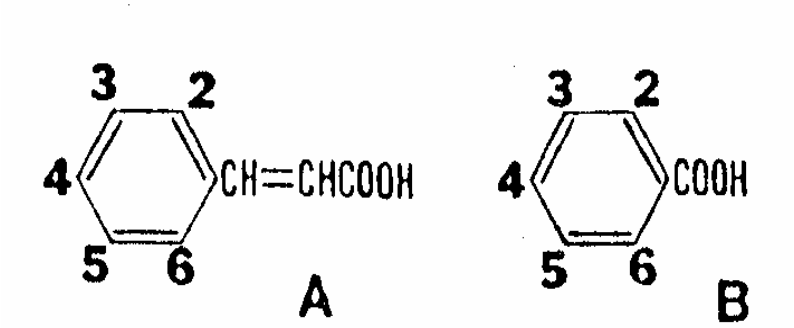

Fig. 2. Basic structure of phenolic acids. A cinnamic acid, B benzoic acid. Ferulic acid (3-methoxy-4-hydroxycinnamic acid) is an example of a cinnamic acid derivative. Vanillic acid (3-methoxy-4-hydroxybenzoic acid) is an example of a benzoic acid derivative.

Flavonoids

Flavonoids form the largest group of phenols in the plant kingdom. The flavonoid compounds consist of two distinct units, a C6-C3 fragment from cinnamic acid which forms the B-ring, and a C-6 fragment from malonyl-Co-A which forms the A-ring (Fig. 3).

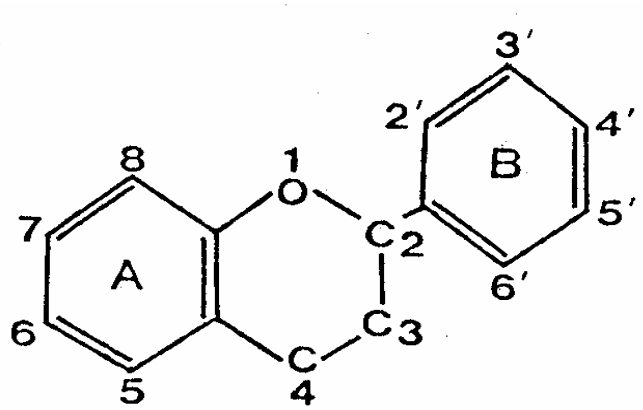

Fig. 3. Basic flavonoid ring structure (Hahn et al 1984).

The major flavonoid groups are: flavanones (carbonyl at 4), flavonols (carbonyl at 4, hydroxyl at 3), flavones (carbonyl at 4, double bond between 2 and 3), and flavans (no carbonyl at 4). The major flavans are leucoanthocyanidins (hydroxyls at 3 and 4), catechin (hydroxyl at 3), and anthocyanidins (hydroxyl at 3, double bond between 3 and 4). The anthocyanidin flavylium ion has a double bond between 3-4 and 1-2, hydroxyl at 3, and positive charge at 1. (Hahn et al 1984).

Anthocyanidins and leucoanthocyanidins often exist as glucosides at 3 or 7 positions and are then referred to as anthocyanins and leucoanthocyanins respectively (Coultate 1996). Anthocyanins are the major color pigments of flowers, stalks and leaves. The anthocyanins are readily converted to their corresponding anthocyanidin in acidic solutions, this makes it difficult to determine whether a pigment is the anthocyanin or anthocyanidin (Hahn et al 1984). Both anthocyanins and anthocyanidins are reported to be present in sorghum. The pericarp color appears to be due to a combination of primarily anthocyanin and anthocyanidin pigments and other flavonoid compounds. The most common anthocyanins in black and other types of sorghums are the 3-deoxyanthocyanidins (Awika et al 2005), and these comprise of glucosides of apigenidin (yellow) and luteolinidin (orange) (Hahn et al 1984). In sorghums with a lemon-yellow pericarp, the major pigment identified was erodictyol (a chalcone), while

the corresponding anthocyanidin, luteoferol was identified as the major pigment in the red pericarp (Hahn et al 1984).

Tannins

Tannins are effectively classified as water soluble phenolic compounds having molecular weights between 500 and 3, 000. Besides giving the usual phenolic reactions, tannins also have special properties such as the ability to precipitate alkaloids, gelatin and other proteins (Haslam 1996). Industrially, tannins are classified as substances of plant origin, which because of their ability to cross-link with protein, are capable of transforming raw animal skins into leather, the tanning process (Harborne 1991, Haslam 1996).

Chemically, there are two classes of tannins, and these are the hydrolysable tannins and condensed tannins. The hydrolysable tannins (eg tannic acid) break down into sugars and a phenolic acid (eg gallic or ellagic) when treated with acid, alkali or some hydrolytic enzymes (tannase) (Harborne 1991). The simplest hydrolysable tannin is pentagalloylglucose, where the glucose molecule has accommodated by esterification, five gallic acid molecules (Waterman and Mole 1994). Sorghum contains condensed tannins or flavolans (Fig. 4). Condensed tannins are formed biosynthetically by the condensation of single catechin (or gallocatechin) units to form dimmers and then higher oligomers, with carbon-carbon linking one flavan unit to the next by a 4-8 or 6-8 link (Harborne 1991, Haslam 1996). Condensed tannins are also referred to as proanthocyanidins because when treated with mineral acids, anthocyanidins are released (Hahn et al 1984). The most common proanthocyanidins are procyanidins, which means that they yield cyanidins or catechin on acid treatment (Harborne 1991). The condensed tannins in sorghum are mainly procyanidins (Gu et al 2004).

Sorghums with more than 1% condensed tannins are regarded as high tannin sorghum (Type III) (Hahn et al 1984). Since tannin content was associated with anti-nutritional qualities of sorghum, rapid methods of identifying high tannin sorghum have been developed. One such method is the bleach test, which is described by Waniska et al (1992). During the Bleach test, the pericarp is dissolved by the bleach reagent, and this

exposes the testa layer, which is black in tannin sorghum, and white to yellow in non-tannin sorghum. Confirmatory or more specific tests can then be done, for example the vanillin-hydrochloric acid method described by Burns (1971).

Determination of phenolic content in sorghum

There are several methods used to determine phenol and tannin content of sorghum, although many methods measure phenolic compounds which may or may not be condensed tannins. Total phenol content can be measured using the Folin Ciocalteau reagent, after extracting the sample using ethanol (Beta 1999). The Folin Ciocalteau assay is based on the reducing power of phenolic hydroxyl groups (Hahn et al 1984). Some methods used are more specific for proanthocyanidins, like the butanol-hydrochloric acid method (Porter et al 1986), and vanillin-HCl method (Burns 1971). The butanol-HCl method is specific for proanthocyanidins which, when treated with mineral acid solutions give colored anthocyanidins with absorbance maxima around 550 nm. The cleavage of the inter-flavanoid bond results in the formation of carbocations, which undergo autooxidation to yield anthocyanidins (Porter et al 1986). In the vanillin-HCl assay, the flavonoid A-ring at the C-6 position reacts with vanillin forming a red chromophore, thus the assay detects any monomeric or polymeric flavanols using catechin as a standard (Beta et al 2000).

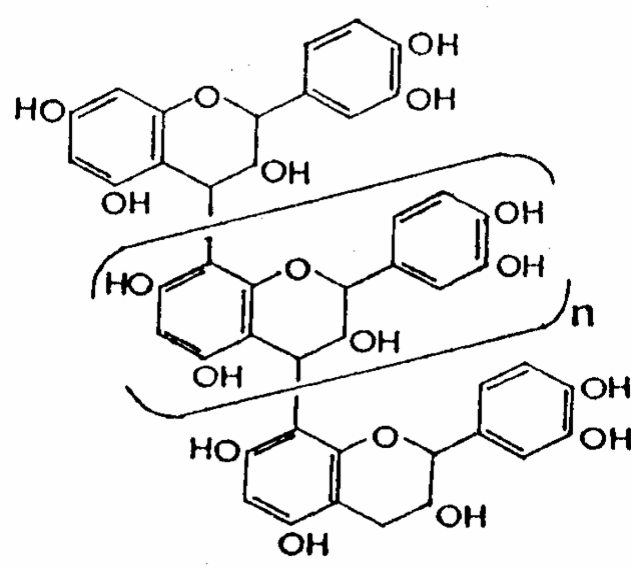

Fig. 4. Structure of proanthocyanidin (tannin) polymer n > 10 (Awika et al 2003b).

Separation and quantification of phenolic compounds using high performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) is a powerful technique that has been used to separate and determine phenolic constituents in fresh fruit products and other samples (Chen et al 2001, Rodriguez-Delgado et al 2001). The other techniques that have been used include UV-Vis spectrophotometry, thin layer chromatography (TLC), gas liquid chromatography (GLC) and more recently capillary electrophoresis (CE) has been shown to be a fast, powerful, clean and efficient separation technique for a wide variety of phenolic compounds (Rodriguez-Delgado et al 2001). The advantage of HPLC over the Folin Ciocalteu method for example, is that HPLC can quantify each phenolic component, as opposed to the general phenolic content. It is important to know the level of the different phenolic compounds because of their different relevance to health (Awika et al 2003b).

In order to separate and quantify phenolic antioxidants in a food product, it is important to prepare the sample appropriately, although there is still controversy as

regards to the best method of sample preparation. For example, in the analysis of wine phenolics, some methods that have been described involve solid-phase extraction of the sample with C_{18} or strong anion exchange (SAX) anionic cartridges, others use liquid-liquid extraction with different organic solvents like ethyl acetate or diethyl ether, and some others involve injecting samples directly without any preparation step (Rodriguez-Delgaldo et al 2001).

Phenolic acids and flavonoids such as anthocyanins are separated by reverse-phase chromatography using C_{18} columns (Lopez et al 2001, Chen et al 2001). The detector used is UV-Vis photo diode array set at 280 nm and 360 nm, and identification is done by matching retention time and spectral characteristics of the flavonoids and phenolic compounds with those of standards (Chen et al 2001). For quantification, standard calibration curves are prepared by plotting the area of peaks against different concentrations of phenolic compound standards (Lopez et al 2001).

Condensed tannins or procyanidin profile in tannin sorghum is determined on normal phase Luna silica columns, after the sample extract has been cleaned of sugars and phenols, using aqueous methanol, on Sephadex LH-20 columns (Awika et al 2003b). The initial separation of procyanidins from the rest of the phenolic compounds is based on the finding that, in alcohol (95% ethanol), tannins were adsorbed on Sephadex LH-20, after which they could be eluted with aqueous acetone (Strumeyer and Malin 1975).

Antioxidant properties of phenolic compounds

Phenolic antioxidants act by donating a proton to a free radical, thus stabilizing it, while the antioxidant free radical generated is stabilized by resonance due to the presence of the benzene ring which allows for the existence of many resonant structures (Coultate 1996) (Fig 5). The free radicals in food systems are usually derived from fatty acid auto-oxidation, which results in chain reactions leading to production of more free radicals. The termination steps produce volatile odorous compounds responsible for the rancid flavor of fatty food. Transition metals, like copper and iron catalyze free radical formation. Donating a proton to the radicals, particularly to the hydroperoxy radicals, breaks these chain reactions, and slows down the rancidity process. The phenolic

antioxidants commonly used in food are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and α-tocopherol (vitamin E) (Moure et al 2001). The latter is natural, while the former two are synthetic. The concerns that have been raised towards the safety of artificial antioxidants have prompted research into antioxidants that occur naturally, like the tannins, flavonoids and phenolic acids in sorghum.

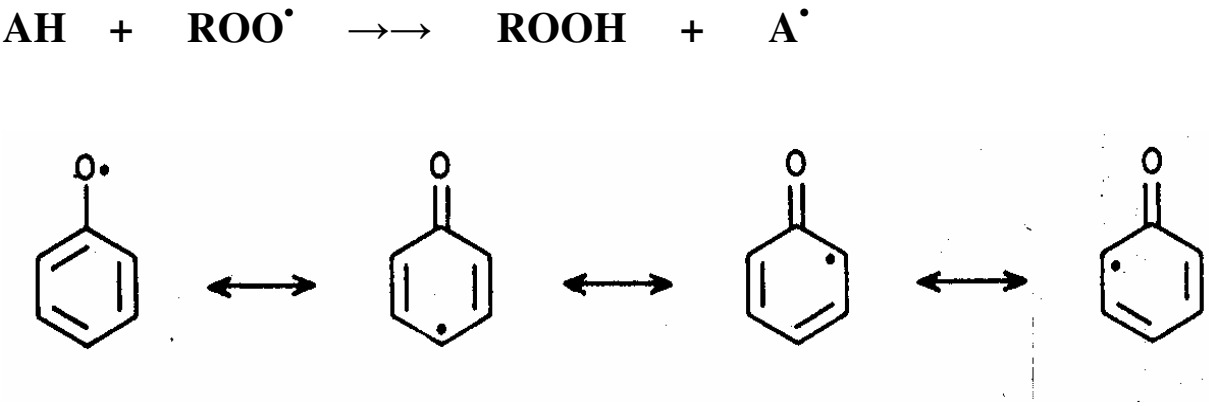

Fig. 5. Activity of antioxidants. The antioxidant (A) donates a hydrogen atom (proton) to the free radical such as ROO•. The antioxidant free radicals are stabilized by resonance and are therefore insufficiently reactive to continue the chain reaction. (Coultate 1996).

The ability of polyphenols to chelate iron and copper further supports their role as preventative antioxidants in terms of inhibiting transition-metal catalyzed free radical formation. Meyer et al (1998) found that the order of activity in the inhibition of copper catalyzed LDL oxidation *in vitro* was catechin > cyanidin ≅ caffeic acid > quercetin > ellagic acid. The antioxidants studied possess a similar 0-dihydroxy moiety. The combination of these polyphenols had additive antioxidant effects except for the combinations including ellagic acid and catechin, ellagic acid seemed to exert antagonistic effects (Meyer et al 1998).

In addition to antioxidant activity, some flavonoids may be able to enhance antioxidant activity by inducing antioxidant enzymes such as glutathione-S-transferase

and superoxide dismutase (Ross and Kasum 2002). *In vitro* studies have demonstrated growth inhibition by procyanidin oligomers of colon, breast and prostatic cancer cell lines (Bawadi et al 2005).

Determination of antioxidant activity

There are several methods used to determine antioxidant activity of a biological material. The most commonly used are those involving chromogen compounds of a radical nature, the presence of an antioxidant leads to the disappearance of these radical chromogens. According to a review by Arnao (2000), the two most widely used chromogen radicals are $ABTS^{\bullet+}$ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) and $DPPH^{\bullet}$ (α,α diphenil-β-picrylhydrazyl radical).

The ABTS radical is used in the Trolox equivalent antioxidant capacity assay (TEAC), where the ability of antioxidants to scavenge the radical cation (ABTS) is measured relative to Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid), a water soluble analogue of vitamin E or ascorbic acid (Re et al 1999). If Trolox is used the antioxidant activity is expressed as Trolox equivalent antioxidant capacity (TEAC). Other tests have been developed that measure scavenging activity with different challengers such as superoxide radical ($O_2^{\bullet}$), hydroxyl ($^{\bullet}OH$), nitric oxide ($^{\bullet}NO$) and alkylperoxyl radicals. Antioxidant activity can also be determined using the modified β-carotene bleaching method (Kaur and Kapoor 2002). The oxidation of the β-carotene emulsion is monitored spectrophotometrically at 470 nm.

The biological antioxidant activity of food extracts can be determined by measuring the inhibition of copper-catalyzed human LDL oxidation *in vitro*, as was done by Meyer et al (1998), when they assessed the antioxidant activity of flavonoids and phenolic acids. In this study, antioxidant activity was monitored by static gas chromatography, which detected the amount of hexanal produced.

Effect of processing sorghum on phenol content and antioxidant activity

Traditionally, the various technologies used in the processing of sorghum into food have focused on improving its nutritional value, particularly in terms of improving protein digestibility (Mukuru 1992). The cause of reduced protein digestibility has been

partially attributed to its ability to bind to tannins and other polyphenols in sorghum (Duodu et al 2002). Technologies such as malting, fermentation and alkaline processing have been used to improve protein digestibility of high tannin sorghum (Monyo et al 1992, Mukuru 1992). Alkaline processing reduces assayable tannins and improves malt quality of high tannin sorghum (Monyo et al 1992 and Beta et al 2000).

The issue of the fate of phenolic compounds and their antioxidant properties during processing has been subject of investigation. A reduction in assayable condensed tannins was observed during the production of fermented sorghum-based non-alcoholic beverage, *Mahewu*, in Zimbabwe (Bvochora et al 1999). The tannins form less extractable polymers either with other food components in particular proteins and carbohydrates or between themselves (Duodu et al 2002). Tannin interactions with food components are mostly non-covalent interactions, and may involve hydrogen bonding and hydrophobic interactions (Asquith and Butler, 1986; Mehansho et al 1987, Renard et al 2001). Sorghum tannins have strong affinity for proteins high in proline content like the prolamins (Emmambux and Taylor 2003). The reduced extractability is observed as reduced measurable levels by methods such as the vanillin-HCl assay (Beta et al 2000).

The possible benefits of naturally occurring antioxidants, as either contributing to food shelf-life stability, and as a source of dietary antioxidants with health benefits has contributed to the growing interest on the effect of processing on antioxidants in food (Dekker et al 1999). Awika et al (2003a) and Dykes et al (2005) showed that in unprocessed sorghum grain, the content of phenolic compounds was a good predictor of antioxidant activity measured by the ABTS and DPPH methods. The effect of sorghum processing on antioxidant properties of African sorghum foods has not been reported.

Bioavailability of dietary phenolic compounds and food processing

The question of bioavailability of dietary polyphenols, especially procyanidins, has been subject of much debate because of their large molecular size. Recent evidence, however suggests that polyphenols may be more bioavailable than previously thought (Rechner et al 2002, Ross and Kasum 2002). *In vitro* studies showed that procyanidins, up to trimers could be absorbed through intestinal cell monolayer (Deprez et al 2001).

Awika et al (2003b) deduced, using HPLC analysis, that there could be fragmentation of procyanidins during extrusion cooking, and it is possible that smaller molecules can be readily absorbed from the intestine, thus increasing bioavailability. Condensed tannins are also relatively unstable molecules and are readily cleaved in mild acid solutions (pH 3-4) to form flavan-3-ol and methide (Porter 1992). Procyanidin oligomers of 3 to 6 flavan units were hydrolyzed to catechin dimers and free catechins in the gastric environment (Spencer et al 2000). The degradation of polyphenols by colonic bacterial enzymes could further increase intestinal absorption (Rechner et al 2002).

The effect of processing on phenolic compounds, in particular sorghum varieties high in procyanidins would further raise the question of bioavailability once the procyanidins have formed complexes or associated with the food matrix. Riedl and Hagerman (2001) demonstrated that tannins, even when bound to protein, were still capable of acting as radical scavengers; this property was regarded as being of particular importance in the gastrointestinal tract where the tannin-protein complexes could act as free radical sinks.

There is need to also investigate the fate of bound tannins once sorghum based food is digested in the gastrointestinal tract. *In vitro* preliminary studies simulating digestion are important because they are less expensive and quick, and they may help provide some insight into the occurrences *in vivo*. The method of Goni et al (1997), for estimating glycemic index in food has been used by several workers and has been found to be highly correlated to data obtained *in vivo*. Thus the method can also be adapted to determine the fate of antioxidants when food is digested or hydrolyzed in the gastro-intestinal tract.

CHAPTER III

EFFECT OF SORGHUM TYPE AND PROCESSING ON ANTIOXIDANT PROPERTIES OF AFRICAN SORGHUM-BASED FOODS

This work determined the effect of sorghum type and different processing technologies of traditional African sorghum foods on total phenols, tannin content and antioxidant activity. The products were prepared by fermentation, conventional and extrusion cooking of whole and decorticated ground grain. The tannin sorghums had higher antioxidant activities, compared to the types without tannins. Antioxidant activity was significantly correlated with total phenols and tannins ($r>0.95$). Decortication reduced antioxidant activity of both tannin and non-tannin sorghum by 82-83% due to removal of the pericarp and the testa, which decreased phenols. Processing generally decreased antioxidant activity, however conventionally cooked porridges had higher antioxidant activity than the extrusion cooked products. The retention of antioxidant activity, particularly in fermented and unfermented porridges, means that whole tannin sorghum can be processed into foods with potential health benefits.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a drought-resistant crop and thus an important food source in semi-arid regions of the world. World production of sorghum is about 57 million tons and ranks fifth in production after maize, rice, wheat and barley (FAOSTAT data, 2005). Some varieties of sorghum are recognized as important sources of dietary antioxidants because of the phenolic compounds found in the grain (Awika and Rooney 2004, Dykes and Rooney 2006).

Phenolic compounds in sorghum occur as phenolic acids, flavonoids and condensed tannins (Serna-Saldivar and Rooney 1995). Condensed tannins (procyanidins) occur in sorghums with a pigmented testa and have dominant B_1B_2 genes (Waniska and Rooney 2000). The tannin sorghums have the highest levels of antioxidants of any cereal

analyzed (Gu et al 2004). Sorghum tannins are 15-30 times more effective at quenching peroxyl radicals than simple phenolics, thus they are potential biological antioxidants (Hagerman et al 1998). Despite their possible beneficial effects as antioxidants, tannins have been linked to reduced protein digestibility of sorghum (Duodu et al 2002), because they complex with proteins and inhibit enzymes (Scalbert et al 2000). The evidence of possible benefits of tannins in the diet has led to research that focuses on sorghum tannins and health.

In many parts of Africa sorghum is milled as whole grain, or decorticated using traditional mortars and pestles or mechanical dehullers (Serna-Saldivar and Rooney, 1995). Decortication removes the grain's outer layers where the polyphenols are concentrated, which reduces overall tannin content (Taylor and Dewar 2001). This improves product color, reduces astringency and improves digestibility. The milled sorghum is used for making fermented or non-fermented soft or stiff porridges. Sorghum is also used in the preparation of malted and fermented beverages such as *Mahewu*, in Zimbabwe (Bvochora et al 1999), or sorghum beer (Taylor and Dewar 2001). Special procedures like an alkali treatment are used to eliminate the negative effect of tannins on sorghum malt enzyme activity (Beta et al 2000).

The food industry in Southern Africa has also been exploring the use of sorghum in the production of ready-to-eat products using extrusion cooking technology and gun-puffing. The tannin sorghums are used in many food products and are sometimes preferred in some areas of Africa. Special products are made from tannin sorghums. Some reports exist on antioxidant activity of fully processed products like cookies and bread containing sorghum bran, as well as extrusion-cooked products (Awika et al 2003a).

Awika et al (2003a) and Dykes et al (2005) showed that in unprocessed sorghum grain, the content of total phenols was an excellent predictor of antioxidant activity measured by the ABTS, DPPH and ORAC methods. Fermentation reduced measurable tannin content of sorghum products (Hassan and El Tinay 1995, Bvochora et al 1999). The reduction in tannins by processing occurs by interaction of tannins with proteins and

carbohydrates (Mehansho et al 1987). These tannin complexes are less extractable, and give reduced tannin levels. Sorghum tannins have strong affinity for proteins high in proline content like the prolamins (Emmambux and Taylor 2003). In aqueous environments, polymerization of tannins also occurs between tannin molecules or with other pigments such as anthocyanins (Remy et al 2000), these complexes, may not be detectable by the common tannin assay methods such as the vanillin-HCl method.

To date, there are limited reports on the effect of different processing methods on antioxidant activity of African sorghum-based foods such as fermented and unfermented porridges. The objective of the study was to evaluate the effect of traditional African processing and extrusion cooking, using a twin-screw cooker extruder, on total phenols, tannin content and antioxidant activities of products from different sorghum types. The products evaluated included porridges and extrudates made from whole and decorticated tannin and non-tannin sorghums.

MATERIALS AND METHODS

Materials

Two non-tannin sorghum types, Macia and NK 283, and three tannin types Red Swazi, NS 5511 and Framida were grown in 2003. Four sorghum types were from Zimbabwe and these were; Macia, Red Swazi, NS 5511 and Framida. NK 283, a non-tannin sorghum type was from South Africa; it has a red pericarp without a pigmented testa. Macia was the white food-type sorghum without a pigmented testa, while Red Swazi was a traditional variety and consisted of mixed red and white grains; over 70% of the kernels had a pigmented testa.

Catechin hydrate and potassium persulfate were obtained from Sigma (St Louis, MO). The Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid) was obtained from Acros Organics (Morris Plains, NJ), while 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from TCI Kasei Koygo (Tokyo, Japan). The Total Starch Assay Kit was obtained from Megazyme International Limited (Ireland).

Analyses

The bleach (chlorox) test

The presence or absence of a pigmented testa in the sorghum grain was determined using the bleach or chlorox test (Waniska et al 1992). The bleach reagent, consisting of 5% sodium hydroxide in 3.5% hypochlorite solution (commercial bleach) was used to dissolve the pericarp layer to expose the testa layer. The kernels of tannin sorghum types, those with a pigmented testa, turned black.

Grain hardness

The hardness of the sorghum kernels was assessed visually using the methods and illustrations in Rooney and Miller (1982). The hardness was recorded using a scale of 1-5, based on the proportion of corneous to floury endosperm, 1 being corneous or hard, and 5, floury or soft. Hardness was then measured using the Single Kernel Hardness Tester (SKHT) Perten SKCS 4100 (Perten Instruments Inc, Chatham, IL), and expressed as Hardness Index (HI).

Grain color

The sorghum kernel color was assessed visually (Fig 6) and also measured using a colorimeter Model CR-310 (Minolta, Osaka, Japan) to obtain the CIE L* values. The L* values provide a measure of the lightness or darkness of the grain, whiter grains have higher L* values, while dark colored or grains with red pericarps have lower L* values.

Proximate composition of the grain

The moisture content of the milled grain was determined by oven drying at 135^{o}C for 2 hours (AACC Method 44-19, 2000). Protein content was determined by combustion analysis using a LECO FP-528 Nitrogen Analyzer (LECO Corporation, St Joseph, MI), while fat content was determined as fat extracted by petroleum ether. Ash content was determined by dry ashing using a muffle furnace at 550^{o}C (AOAC Method 923.03, 2002). Starch was determined by the AACC Method 76-13 (2000) using the Megazyme Total Starch Assay Kit. The Megazyme method is based on the conversion of starch to glucose using thermostable α-amylase and amyloglucosidase.

Determination of total phenols

The modified Folin Ciocalteu method of Kaluza et al (1980) was used to quantify total phenols. The milled samples were extracted for 2 hr using acidified methanol (1 % HCl in methanol), and shaking was done at low speed in an Eberbach shaker (Eberbach Corp., Ann Arbor. MI). The extracts were centrifuged and the supernatant used for total phenols quantification. An aliquot of the extract (0.1 mL) was diluted with 1.1 mL water, and then reacted with 0.4 mL Folin Ciocalteu reagent and 0.9 mL of 0.5 M ethanolamine. The reaction was carried out for 20 min at room temperature and absorbance was read at 600 nm. The standard used was catechin; total phenols were expressed as mg catechin equivalents per g (mg CE/g).

Determination of tannin content

Tannin content was determined using the vanillin-HCl method as described by Price et al (1978). The milled samples were extracted at 30^{o}C for 20 min using acidified methanol. Supernatants were obtained by centrifuging the extracts, and 1 mL aliquots were mixed with 5 mL vanillin reagent, and absorbance read at 500 nm after 20 min Blank determinations were done to counteract the effect of anthocyanins and other pigments in the samples. The standard used was catechin; tannin content was expressed as mg catechin equivalents per g (mg CE/g).

Antioxidant activity assay

Antioxidant activities of the sorghum extracts were determined using the ABTS and DPPH methods as described by Awika et al (2003a). For the ABTS assay, the sample extracts were prepared by extracting the milled sample for 2 hr with acidified methanol. For the DPPH assay the samples were extracted in 70% aqueous acetone. The standard used was Trolox, and antioxidant activity was reported as μmol Trolox Equivalent Antioxidant Capacity per g (μmol TE/g).

The ABTS radical was generated for over 12 hr in the dark, by reacting equal amounts of 8 mM ABTS solution with 3 mM potassium persulfate. The ABTS free radical mixture was diluted using pH 7.4 phosphate buffer; 5 mL of the free radical mixture was diluted with 145 mL phosphate buffer. The diluted ABTS radical was

reacted with the sorghum extracts for 30 min, and absorbance measured at 734 nm (Fig. 7).

The DPPH (1,1-Diphenyl-2-picryl-hydrazyl) reagent was dissolved in methanol and kept at –20^{o}C in the dark prior to use. The DPPH radical was reacted with sorghum extracts for 6 hours, after which absorbance was measured at 515nm.

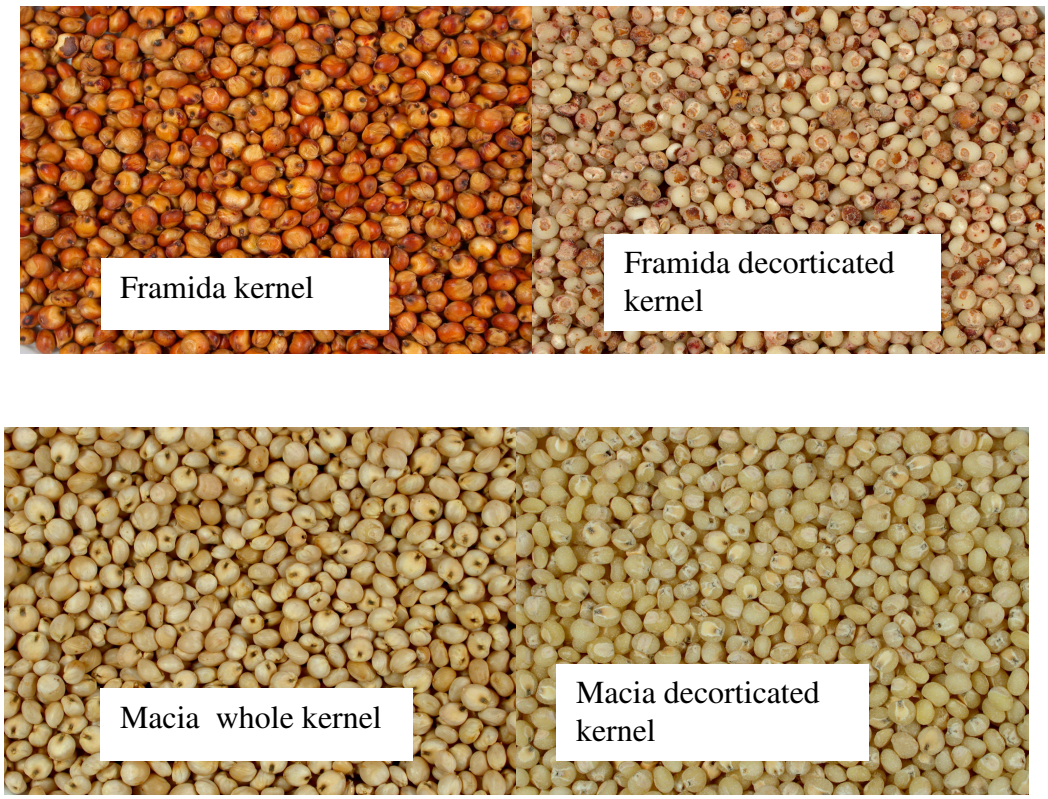

Fig. 6. Whole and decorticated Type III (Framida) and Type I (Macia) sorghum kernels.

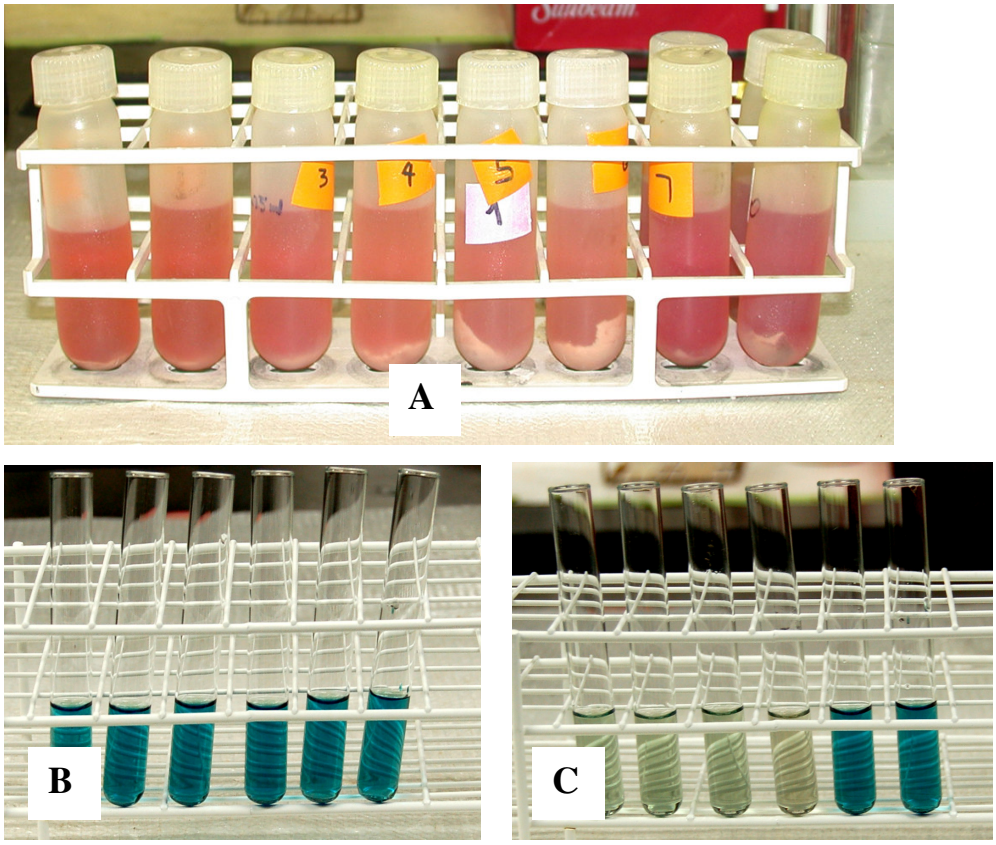

Fig. 7. Sorghum extracts in acidified methanol (A), ABTS free radical mixture (B), ABTS after decolorization by the sorghum extract (C).

Sorghum processing

Decorticating and milling the grain

The sorghum grain was decorticated for 6-8 min to obtain extraction rates of 70-81% using a PRL dehuller (Rural Industries Innovation Center, Kanye, Botswana). The PRL (Prairie Research Laboratory) dehuller abrades off the pericarp and testa layers using rotating resinoid discs (Taylor and Dewar 2001). The soft endosperm varieties, such as the tannin sorghums, Framida and NS 5511, were decorticated for 6 min to avoid large losses due to endosperm fragmentation. The whole and decorticated grain was milled using a hammer mill.

Fermentation and preparation of the porridges

A traditional lactic acid starter culture was prepared by back slopping as described by Taylor and Taylor (2002). The starter was prepared from the natural micro-flora on the grain using decorticated, milled NK 283. The preparation involved suspending 100 g of flour in 90-100 mL boiled, cooled water, leaving the slurry at 25^{o}C, until pH dropped to 3.6, after about 48 hr. A portion (20 mL) of the fermented slurry was transferred to a fresh batch of sorghum flour slurry and the whole mixture was fermented at 25^{o}C for seven days. This procedure was repeated to maintain a natural inoculum.

The bulk starter culture was prepared by inoculating 500 g flour with the lactic acid starter, and then fermenting to pH 3.6. Portions (20 mL) were taken from the bulk starter and then added to 235 g milled grain suspended in 300 mL water, and the mixture fermented for 24 hr or until pH dropped to 3.6. The fermentation pH was determined at regular intervals, and the soured slurry was mixed with 1 L boiling water and cooked with constant stirring for 10 min. The unfermented porridge was prepared by suspending the sorghum flour in cold water, and then adding boiling water and cooking as described for the fermented porridge. The fermented slurry and porridges were freeze-dried and analyzed for total phenols, tannins and antioxidant activity.

Extrusion cooking

Whole and decorticated sorghum was coarsely milled using to screen size 1.58 mm using a Hippo hammer mill (Precision Grinders Engineers, Harare, Zimbabwe). The milled grain was extruded in a Clextral BC92 twin-screw, co-rotating extruder (Clextral, FIRMINY Cedex, France). The feed rate was 550 kg/ hr, and moisture content of feed adjusted to about 18%, by injecting water at 45 L / hr. The screw rotation speed was 230 rpm, and barrel temperature was maintained at 150 and 160^{o}C, and residence time was 30-90 sec. The die diameter was 2 mm and the cutter speed set at 120 rpm. After extrusion, the sorghum extrudates were placed in containers and allowed to cool and equilibrate for a few hours (4-5 hr).

Sample preparation

The processed and unprocessed sorghum samples, including the freeze dried fermented slurries and porridges, were milled to pass through a 1 mm screen using the UDY cyclone mill Model 3010-030 (UDY Corporation, Fort Collins, CO.).

Statistical analyses

The statistical software SPSS version 11.5 (SPSS Inc. Chicago, IL) was used. Mean values of data were analyzed with one-way analysis of variance (ANOVA). The means were separated using Fisher's least significant difference (LSD) at $P<0.05$. The correlations of total phenols and tannins with ABTS antioxidant activity, and that of ABTS and DPPH antioxidant activities were determined using Linear Regression.

RESULTS

Grain quality

The sorghum types Red Swazi, NS5511 and Framida had pigmented testa layer (tannin sorghum) (Table I), although Red Swazi was a blend of non-tannin and tannin sorghums. Macia and NK 283 were Type I sorghums without a pigmented testa (non-tannin sorghum). The tannin sorghums had darker colored grains (L* of 45 to 49), while Macia was the lightest in color, with the highest L* value (67), and NK 283, with a red pericarp had an L* value of 50.

The kernels of Macia and NS 5511 were 32 and 31 mg in weight respectively, while those of Red Swazi and NK 283 were smallest, with an average weight of about 25 mg. The hardness of the kernels was significantly different. The non-tannin Macia, had the hardest kernels, with hardness index (HI) of 85, while the tannin sorghum Framida, with a floury endosperm, was soft, with an HI of 44.

The starch, protein, fat and ash contents of the sorghum types are shown in Table II. The starch contents of Macia, Red Swazi and Framida were lower (73-75%), than those of NS 5511 and NK 283 (80-82.6%). The protein content range was 8.2-12.8%, with Macia having the highest protein content, and Framida the lowest. The fat and ash contents ranged from 2.7 to 3.7%, and 1.5 to 1.6% respectively.

Table I
Effect of sorghum type on grain quality of Macia, NK 283, Red Swazi, NS 5511 and Framida

Sorghum type	Pericarp color	L* value	Bleach Test[a]	Kernel hardness score[b]	Kernel hardness index (HI)[c]	Kernel weight (mg)[c]	Kernel diameter (mm)[c]	% Decorticated grain yield
Macia	White	67.0 a	0	2.0	84.6 a	32.0 a	2.3 a	81
NK 283	Red	50.2 b	1	3.0	72.9 b	24.9 c	2.1 b	71
Red Swazi[d]	Red	49.0 c	77	3.5	63.7 d	24.7 c	2.2 b	75
NS 5511	Red	45.7 d	99	5.0	68.6 c	31.4 a	2.4 a	70
Framida	Red	45.8 d	99	5.0	44.0 e	29.6 b	2.2 b	72

[a]Number of kernels, out of a 100, with pigmented testa layer
[b]Visual kernel hardness evaluated using a score of 1 to 5: 1 is hard and 5 is soft or floury endosperm (Rooney and Miller 1982)
[c]Kernel hardness, weight and diameter determined using the Single Kernel Hardness Tester (Perten SKCS 4100)
[d]Red Swazi, was a mixture of red and white grains
Values within the same column with different letters are significantly different at $P<0.05$.

Table II
Proximate composition (%) (dry basis) of Macia, NK 283, Red Swazi, NS 5511 and Framida

Sorghum type	Moisture	Starch content	Protein content (N=6.25)	Fat content	Ash
Macia	11.4	73.3 c	12.8 a	2.7 c	1.5 ab
NK 283	10.6	82.6 a	10.8 b	3.7 a	1.5 b
Red Swazi	11.4	75.6 c	10.4 c	3.3 b	1.6 ab
NS 5511	11.4	80.8 b	10.0 d	3.0 b	1.5 ab
Framida	11.9	73.5 c	8.2 e	3.7 a	1.5 ab

Values within the same column with different letters are significantly different at $P<0.05$.

Effect of sorghum type, decortication and different processing methods on total phenols

The total phenols were significantly affected by sorghum type, decortication and processing (Table III). The sorghum types with pigmented testa layers (condensed tannins) had higher levels of total phenols [19.7 to 24.5 mg catechin equivalents per g (mg CE/g)], than the types without a pigmented testa (2.7-5.3 mg CE/g). The total phenol levels differed significantly ($P<0.05$) between the different sorghum types, with the tannin sorghums having the highest, followed by NK 283 and lastly Macia.

Decortication significantly reduced the level of total phenols, when compared with the whole grain, and the tannin sorghums had greatest reductions. The extent of reduction of total phenols, on decortication, corresponded to decorticated yield (R-square =0.65). Macia, with the highest yield (81%), had total phenols reduced by 33%, while NS 5511, with 70% decorticated yield, had 77% reduction of total phenols.

The fermented slurries of both whole and decorticated tannin sorghums had significantly reduced total phenols, when compared to the respective unprocessed grain fractions (whole grain and decorticated grain). The fermented porridges from whole tannin sorghum showed a further reduction in total phenols, when compared to the fermented slurries (uncooked). Extrusion cooking also significantly reduced measurable total phenols for both whole and decorticated tannin sorghums. Fermentation, porridge making and extrusion cooking did not significantly affect the level of total phenols in processed NK 283 and Macia.

Table III
Effect of different processing methods on total phenols of sorghum

Sample	Raw grain		Fermented slurry		Fermented porridges		Extrusion cooked sorghum	
	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated
Macia	2.7 k	2.2 l	2.2 l	2.5 k	3.3 k	2.9 k	1.8 l	2.3 l
NK 283	5.3 j	2.0 l	2.8 k	2.6 k	4.4 j	3.0 k	2.3 k	3.4 j
Red Swazi	19.7 c	6.6 h	9.5 f	4.3 j	8.7 g	[b]NR	6.0 h	2.5 k
NS 5511	22.4 b	4.7 j	10.1 e	5.2 j	9.1 g	3.6 j	6.7 h	4.1 j
Framida	24.5 a	8.5 g	16.3 d	7.5 h	NR	NR	5.3 i	3.7 j

[a] Total phenols expressed as mg Catechin equivalents per g sample, dry basis (Folin Ciocalteu method)
[b] NR- no data value
[c] Data values with different letters are significantly different at $P<0.05$

Effect of sorghum type, decortication and different processing methods on tannin content

The sorghum types without a pigmented testa, Macia and NK 283 did not have detectable tannins, while sorghum types with a pigmented testa, Red Swazi, NS 5511 and Framida, had tannin levels ranging from 33.6 to 49.1 mg CE / g (Table IV). Red Swazi had significantly lower tannin content that the other two tannin sorghums.

Decorticating the grain significantly reduced tannin content, by as much as 79-92%. The tannin contents of decorticated NS 5511 and Red Swazi were not significantly different, 3-4 mg CE/g, respectively, while that of decorticated Framida was significantly higher (9.7 mg CE/g). Decorticated grain yields did not appear to have affected the retention of tannins, although the decorticated yield of Framida was slightly higher than that of NS 5511.

The fermented whole tannin sorghum slurries and porridges had significantly reduced measurable tannin levels. The decorticated fermented slurries and porridges of Red Swazi and NS 5511 did not have detectable tannins. The fermented slurry of decorticated Framida had low tannin content (3.9 mg CE/g), a reduction of 60%, when compared to the decorticated unprocessed grain. Extrusion cooking reduced measurable tannins by almost 97% in whole grain extrudates, while in the decorticated extrudates, they were not detected. Tannin levels were thus significantly reduced by processing; cooking, both conventional and extrusion had the most adverse effects.

Table IV
Effect of different processing methods on tannin content of sorghum

Sample	Raw grain		Fermented slurry		Fermented porridges		Extrusion cooked sorghum	
	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated
Macia	[b]ND	ND	ND	ND	ND	ND	ND	ND
NK 283	ND	ND	ND	ND	ND	ND	ND	ND
Red Swazi	33.6 b	3.4 g	11.4 e	ND	2.0 h	NR	0.9 h	ND
NS 5511	49.1 a	4.2 g	15.5 d	ND	4.5 g	ND	1.9 h	ND
Framida	47.8 a	9.7 f	24.0 c	3.9 g	[c]NR	NR	0.4 h	ND

[a] Tannin content expressed as mg Catechin equivalents per g sample, dry basis (Vanillin-HCl method, blanks subtracted)
[b]ND-not detected
[c] NR- no data value
[d] Data values with different letters are significantly different at P<0.05

Effect of sorghum type, decortication and different processing methods on antioxidant activity

Antioxidant activity was significantly affected by sorghum type and processing method (Table V). The tannins sorghums, Red Swazi, NS 5511 and Framida, had significantly higher antioxidant activity when compared to sorghums without a pigmented testa layer, Macia and NK 283. Framida had the highest ABTS antioxidant activity [427 μmol Trolox equivalents per g (μmol TE/g)], while Macia had the lowest (22 μmol TE/g). The sorghum types studied had significantly different antioxidant activities.

Decortication significantly reduced antioxidant activities of both tannin and non-tannin sorghums. Antioxidant activity was reduced by 73 to 87 %. Antioxidant activity of Framida was reduced from 427 to 93 μmol TE/g, while that of Macia was reduced from 22 to 6 μmol TE/g. The antioxidant activities of decorticated Red Swazi and NS 5511 were not significantly different from that of whole NK 283.

The whole fermented slurries of tannin and non-tannin sorghums had reduced measurable antioxidant activities when compared to the raw grain. Preparation into fermented porridges further reduced the antioxidant activities of whole tannin sorghum porridges, while those of non-tannin sorghums did not change significantly. As expected, decorticated fermented porridges had significantly lower antioxidant activity than whole grain porridges. Extrusion cooking also significantly reduced measurable antioxidant activity, by up to 86%, when compared to that of the unprocessed grain. The antioxidant activities of extrudates produced from decorticated NK 283, Red Swazi, NS 5511 and Framida, did not differ significantly.

Table V
Effect of different processing methods on antioxidant activity of sorghum

Sample	Raw grain		Fermented slurry		Fermented porridges		Extrusion cooked sorghum	
	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated	Whole	Decorticated
Macia	22 i	6 j	6 j	1 j	6 j	3 j	4 j	4 j
	(3)	(3)	(6)	(1)	-	-	(5)	(7)
NK 283	52 c	7 j	25 i	3 j	20 i	4 j	12 ij	18 i
	(14)	(2)	(16)	(6)	-	-	(12)	(15)
Red Swazi	359 c	51 h	102 f	15 ij	68 g	[b]NR	48 h	13 ij
	(182)	(35)	(61)	(15)	-	-	(63)	(13)
NS 5511	384 b	49 h	147 e	20 i	74 g	13 ij	58 gh	22 i
	(244)	(29)	(70)	(16)	-	-	(64)	(25)
Framida	427 a	93 f	200 d	46 h	NR	NR	53 h	19 i
	(305)	(61)	(91)	(35)	-	-	(68)	(26)

[a] ABTS antioxidant activity expressed as µmol Trolox equivalent antioxidant capacity per g sample (µmol TE/g), dry basis. (Values in parentheses are the DPPH antioxidant activity values).
[b] NR- no data value
[c] Data values with different letters are significantly different at P<0.05
- No data value

DISCUSSION

Tannin sorghums were softer than non-tannin sorghums, an observation similar to that of Beta et al (1999). Tannin sorghums gave lower decorticated yield (average 72%) than Macia (81%) because the kernels were softer and thus fragmented easily, resulting in endosperm loss with the bran. Sorghum kernels with a high proportion of hard endosperm are suited for dehulling by pearling procedures (Beta et al 1999), and give higher yield of decorticated product. The starch, protein, fat and ash contents of the sorghum types were within the normal ranges reported for sorghum (Rooney and Waniska 2000).

The sorghum with pigmented testa layers had the highest total phenols and antioxidant activity than non-tannin sorghum types. Dykes et al (2005) and Dicko et al (2005) reported similar results. The total phenols and tannin content of sorghum and the processed products (porridges and extrudates) were highly correlated to ABTS antioxidant activity (R-square = 0.96 and 0.94 respectively). Antioxidant activity determined by the ABTS method was higher than that determined by DPPH method, although the two methods were highly correlated, (R-square = 0.93). The lower values of the DPPH assay are probably due to interference by sorghum grain pigments at 515 nm, the wavelength used to measure decolorization of the DPPH radical (Arnao 2000). The wavelength used for the ABTS assay (734 nm) is outside the visible range, and there is minimum interference by colored pigments such as anthocyanins and carotenoids (Arnao 2000). Awika et al (2003a) demonstrated that the ABTS and DPPH methods were highly correlated with the oxygen radical absorbance capacity (ORAC) method, which uses biologically relevant free radicals such as peroxyl ($ROO^{\bullet}$) and hydroxyl ($OH^{\bullet}$) radicals.

Decortication reduced total phenols, tannins and antioxidant activity because the process removes the grain's pericarp and part of the testa layer where the polyphenols are concentrated (Hahn et al 1984). Both ABTS and DPPH antioxidant activities were reduced by 67 to 88 % for the decorticated grain. Decortication is favored in the production of tannin sorghum products because it reduces astringency, associated with

tannin content, and produces lighter colored products (Taylor and Dewar 2001). Product color has been cited as one of the major quality criteria of sorghum-based products (Kebakile et al 2003), and lighter colored products, like soft and stiff porridges are favored in most parts of Africa (Beta et al 1999). With respect to tannin and red sorghums, a reduction in color could be associated with reduced potential benefit from the phenolic antioxidants.

The reductions in measurable or *in vitro* antioxidant activities of fermented slurries (or fermented milled grain) and porridges were linked to reduced measurable total phenols and tannins. The observed reduction in measurable tannin levels confirmed studies where fermentation reduced tannin levels, and improved *in vitro* digestibility of protein (Hassan and El Tinay 1995, Osman, 2004). Hassan and El Tinay (1995) reported tannin reduction of 61 to 63%, while Osman (2004) found tannin reductions ranging from 15 to 35%. In our study, measurable tannins in whole fermented slurries were reduced by 49 to 68%. Several conflicting explanations were given for the apparent reduced tannin content after fermentation. One suggestion is that in aqueous environments, tannins bind with protein and other components, reducing their extractability (Scalbert et al 2000). Another explanation is that, during fermentation tannins may be degraded by microbial enzymes (Towo et al 2006). Polyphenol oxidase (PPO) reduced phenolic content in tannin sorghums (Towo et al 2006).

In plants, PPOs may synergistically act with peroxidases (POX) during enzymatic browning. PPO and POX activity was detected in the leaves and grains of sorghum; generally, red sorghum varieties with high phenolic content had high PPO and low POX activities (Dicko et al 2002). Thus, PPO activity, either from the cereal grain or the fermenting microorganisms may cause a decrease in phenolic compounds of fermented products. The role of PPO activity could be used to explain the lower tannin content of fermented whole NS 5511 porridge (5 mg CE/g), when compared to that of unfermented porridge [7 mg CE/g (data not shown)]. In contrast, the total phenols and antioxidant activities of these two types of porridges were not significantly different.

Extrusion cooking significantly reduced ABTS antioxidant activity by 83% to 87%, for the sorghum products compared to the raw grains. This was expected since measurable total phenols and tannins were also significantly reduced. Thus only 13 to 23% of antioxidant activity of the original raw or unprocessed grain was extracted from the product. These retentions are significantly lower when compared to those of sorghum extrudates reported by Awika et al (2003a), where 70-100% of antioxidant activity was retained. Awika et al (2003a) used a single-screw friction type extruder as compared to the twin-screw steam heated extruder used in our study. We used a co-rotating twin-screw cooker extruder and the grain was coarsely-milled, and then tempered to about 18% moisture prior to extrusion. Awika et al (2003a) did not temper the sorghum and extruded whole kernels containing 11.5-12.5% moisture. The smaller fraction size that was used in this study probably increased surface area of contact between grain components and promoted intimate interactions during extrusion. The higher moisture content also promoted phenolic and tannin polymerizations (Remy et al 2000), thus the observed reduced extractability of total phenols and tannins, and lower antioxidant activity.

The operating systems of twin-screw and single-screw friction-type extruders differ, in the friction-type single-screw extruder the food material is transported by the rotation of the single screw, and friction generates heat that cooks the sorghum. In the twin-screw extruder there are two rotating screws, and the material is conveyed by alternate transfer from one screw to the other, this promotes mixing and enhanced heat transfer to viscous food components (van Zuilichem et al 1999). The twin-screw extruder has a low shear level, which allows the survival of starch granules that are important for water-absorption of the product (van Zuilichem et al 1999). Mixing and low shear may enhance chances of molecular interactions particularly with respect to interactions of condensed tannins with protein.

The single-screw extruders are suitable for highly expanded products and melt-in the mouth texture, because of the low moisture (<15%), high shear and high temperature extrusion conditions in which significant starch damage occurs. The high amount of

friction and shear may cause depolymerization of the condensed tannins and their conversion to low molecular weight oligomers that are more extractable (Awika et al 2003b). The observed higher antioxidant activity of extruded decorticated NK 283 (18 μmol TE/g), when compared to raw decorticated grain (7 μmol TE/g) could be due to increased extractability of phenols, as well as preliminary products of Maillard browning reactions which are known to generate compounds with antioxidant activity.

The application of heat and moisture either in the preparation of porridges, or during extrusion cooking resulted in significant reductions in antioxidant activity, the relative reductions were higher for tannin sorghums compared to non-tannin sorghum types. The significant reduction in antioxidant activity in cooked high tannin sorghum can be attributed largely to interaction of tannins with prolamins (Emmambux and Taylor 2003). It can be inferred that, protein denatured by cooking had open loose structures which promoted tannin-protein interactions. Riedl and Hagerman (2001) found that tannin-protein complexes retained their antioxidant activity, and thus had potential to act as free radical scavengers in the gastrointestinal tract. Studies have demonstrated *in vitro* breakdown of large tannin polymers by microbial flora in the colon (Deprez et al 2000); thus it is possible that even the protein-tannin polymers may be broken down, and the tannins released.

Overall, decorticating the sorghum grain and extrusion cooking had the greatest impact on antioxidant activity. Even though processing reduced antioxidant activity, whole tannin and non-tannin sorghum products retained higher antioxidant activity than those from decorticated grain. In the ready-to-eat products, the porridges retained higher antioxidant activity than the extrudates. These results demonstrate that the antioxidants present mainly in the pericarp layer, are some of the added benefits of consuming whole grain foods.

CHAPTER IV

PROCYANIDINS IN PROCESSED SORGHUM-BASED PRODUCTS

The processing of tannin-containing sorghum grains may alter the profile of extractable and thus measurable procyanidins (condensed tannins) in sorghum-based foods. This has an effect on the biological properties of the procyanidins such as astringency, enzyme inhibition and antioxidant activity. The objective of study was to quantify the procyanidin profile in sorghum-based porridges, extrudates and bread containing tannin sorghum bran. The amount of procyanidins quantified using normal-phase HPLC with fluorescence detection were compared to tannin content and total phenols determined using the vanillin-HCl and Folin Ciocalteu assays respectively. Processing decreased measurable procyanidins compared to the unprocessed grain; the bread containing bran had the highest decrease, followed by extrudates and porridges. The extractability of polymers (DP>8) was the most affected, while that of oligomers (DP 2-8) and monomers in porridges was not significantly changed. Thus, in processed foods there is potential health benefit from procyanidin oligomers and monomers.

INTRODUCTION

Procyanidins (PCs) are found in sorghum varieties that have a pigmented testa, and classified as either Type II or Type III sorghums (Waniska and Rooney 2000). The procyanidins (condensed tannins) consist of catechin and epicatechin monomer units (Gu et al 2004). They are among the most abundant polyphenolic compounds in plants. Procyanidins are found in various fruits, legumes, some cereal grains, chocolate and beverages like fruit juices and beer (Deprez et al 2000, Hammerstone et al 2000, Gu et al 2004, Awika and Rooney 2004). Among the cereal grains, some varieties of sorghum and barley contain condensed tannins; sorghum has the highest amounts (Gu et al 2004).

Sorghum procyanidins have attracted attention due to their potential health benefits, mainly as dietary antioxidants, and thus could be important in the protection of the body from damage induced by oxidative stress (Awika and Rooney 2004). Oxidative

stress is implicated in most chronic diseases such as coronary heart disease and some types of cancers (Rice-Evans 2001). Although most studies on procyanidins are based on *in vitro* and animal studies, long-term consumption of foods rich in catechin and procyanidins has been associated with reduced risk of cardiovascular disease and cancer. Recently procyanidins from black beans were found to inhibit growth of colon, breast and prostatic cancer cells (Bawadi et al 2005). Black bean procyanidins were found to consist of a mixture of trimers and tetramers, the monomeric unit was either catechin or epigallocatechin (Bawadi et al 2005).

Procyanidins are mainly characterized by their hydroxylation patterns, stereochemistry, the proportions of flavan-3-ol units, and by their degree of polymerization (DP) (Guyot et al 2001). The most important feature of PCs is their degree of polymerization, since it affects their ability to associate with proteins and polysaccharides (Guyot et al 2001), and their bioavailability. PCs with high degree of polymerization may interact with proteins and carbohydrates during processing (Mehansho et al 1987). The complexes formed are less extractable, and give reduced tannin levels. In aqueous environments, the PCs polymerize with other tannin molecules or with other pigments such as anthocyanins (Remy et al 2000). If these complexes are formed in processed food; procyanidins may not be detected by the common assay methods such as the vanillin-HCl method.

Since procyanidins are reactive in the food matrix, their accurate characterization and quantification in processed foods has always been a challenge. In recent years modifications to the normal phase HPLC methods has allowed separation of oligomers up to decamers (DP 10), and polymers were resolved as one peak (Gu et al 2002). Awika et al (2003b), using normal-phase HPLC, quantified and evaluated procyanidins in sorghum grain, some baked products, as well as extrudates produced using a single-screw friction type extruder. They reported reduced measurable procyanidins in the processed sorghum products; they observed a higher proportion of low molecular weight oligomers in the extruded grain.

Although reports of the procyanidin profile of processed sorghum foods exist, to our knowledge there is no report of a comparative study involving the production of porridges from different types of tannin sorghums, with other products such as extruded grain. Porridges, either soft or stiff are popular ways of consuming sorghum (Serna-Saldivar and Rooney 1995). Twin-screw extrusion cooking is favored in the production of ready-to-eat breakfast cereals because of the lower levels of shear than those in friction type single-screw extrusion. Low shear results in less starch damage, which is important for water absorption properties of the product (van Zuilichem et al 1999).

The objective of the present study was to evaluate the effect of processing tannin sorghum on procyanidins. Extrudates were produced using single-screw-friction type extrusion and twin-screw extrusion cooking. The amount of procyanidins quantified using HPLC was compared to tannins and total phenols determined using the vanillin-HCl and Folin Ciocalteu methods respectively.

MATERIALS AND METHODS

Sorghum samples

Grain of three tannin sorghums grown in 2003 were obtained from Zimbabwe and Texas A&M University, College Station, Texas. The tannin sorghums from Zimbabwe were NS 5511 and Framida, and that from Texas was Early Sumac.

Tannin sorghum bran was obtained by decorticating tannin sorghum grain (ATx 623 x RTx SC103 College Station, Tx 2003), in 4-kg batches in a PRL mini-dehuller (Nutama Machine Co., Saskatoon, Canada). The bran was then separated with a KICE grain cleaner (Model 6DT4-1, KICE Industries Inc., Wichita, KS). The bran (approx. 18% of original weight) was further milled into a fine powder.

Gallic acid, catechin hydrate and Sephadex LH-20 were obtained from Sigma (St.Louis, MO). The procyanidin standards were prepared from the tannin sorghum, Sumac, grown in 2003, and the procedure of Awika et al (2003b) was followed. All the solvents were HPLC grade. The water used was double distilled and de-ionized tap water.

Sorghum processing

Preparation of the sorghum porridges

The whole grains of sorghum were milled and then prepared into porridges. The milled grain (78.3 g) was added to 100 mL water; the slurry was added to 333 mL of boiling water and cooked with constant stirring for 10 min. The porridge was cooled for 30-60 min, and frozen using liquid nitrogen and freeze-dried. The freeze-dried samples were stored at -20^{o}C until analysis.

Extrusion cooking of sorghum

The NS 5511 and Framida whole sorghum grains were coarsely milled using a Hippo hammer mill (Precision Grinders Engineers, Harare, Zimbabwe), to pass through screen 1.58 mm, and then extruded in a Clextral BC92 twin-screw, co-rotating extruder (Clextral, FIRMINY Cedex, France). The feed rate was 550 kg/hr; moisture content of feed was adjusted to 18%, by injecting 45 L water per hr. The screw rotation speed was 230 revolutions per min (rpm), and barrel temperature maintained at 150 and 160^{o}C, and residence time was 30-90 sec. The die diameter was 2 mm and the cutter speed set at 120 rpm. After extrusion, the sorghum extrudates were placed in containers and allowed to cool and equilibrate 4-5 hr. The final moisture content was 6-8%.

The Early Sumac extrudates were prepared according to the method described by Perez, (2005). The whole grains were tempered to 14% moisture, and then cracked into ½-1/8 pieces in an attrition mill of (Glenn Mills Inc., Maywood, NJ). The cracked grain was extruded using a single-screw friction-type extruder (Model MX-3001, Maddox, Dallas, Tx). The extruder was preheated to 150^{o}C, and the product cooked by heat generated by friction. The feed rate was 158.8 kg/hr, and screw rotation speed was 341 rpm. The die diameter was 3.18 mm. After extrusion the extrudates were dried at 100^{o}C for 30 min; final moisture content was 3.5%.

Preparation of bread and dough containing tannin sorghum bran

Wheat bread containing 12% tannin sorghum bran was prepared using bakery patent wheat flour (Gold Medal Superlative, General Mills Inc., Minneapolis, MN), using a modified method of Gordon (2001). The bread and dough were prepared in a

bread machine TR800 Breadman Plus automatic bread maker (Salton/MAXIM Housewares Inc., Mt Prospect, IL). The wheat flour used was 459.1 g and the setting on the bread machine was for a 680 g loaf of bread. The ingredients were (as Baker's %): flour, 100; water, 62.0; sugar, 6.0; salt, 1.5; active dry yeast, 0.8; sodium stearoyl lactylate (SSL), 0.3; oil, 3.0; tannin sorghum bran 12.0; vital gluten, 1.0. The total preparation time for the bread (kneading, proofing and baking), was 3 hr; the baking time was 50 min.

Dough samples were taken after dough development and proofing prior to baking. The dough was frozen using liquid nitrogen, and freeze dried. The freshly baked bread was cooled, and freeze-dried in similar fashion. The freeze-dried dough and bread samples were stored at -20^{o}C in airtight packages until analysis.

Sample preparation

The sorghum samples and processed products, including the freeze-dried products, were milled to pass through a 1 mm screen using a UDY cyclone mill (Model 3010-030, UDY Corporation, Fort Collins, CO.).

Analysis

Determination of total phenols and tannin content

The total phenols were determined using the modified Folin Ciocalteu method (Kaluza et al 1980). The milled sorghum grain and the freeze-dried processed samples were extracted using 1% HCl in methanol. A 0.1 mL volume of the extract was diluted with 1.1 mL water, and then reacted with 0.4 mL Folin Ciocalteu reagent and 0.9 mL 0.5M ethanolamine. The reaction was 20 min at room temperature; absorbance was read at 600 nm. Gallic acid was used as a standard; total phenol content was expressed as mg gallic acid equivalents per g (mg GAE/g).

The tannin content of the samples was determined using the modified vanillin-HCl method (Price et al 1978). The samples were extracted for 20 min at 30^{o}C using 1% HCl in methanol, and then centrifuged. A 1 mL volume of the supernatant was mixed with 5 mL vanillin reagent, and absorbance read at 500 nm after 20 min. Blank determinations were done to compensate for the color of the samples, by replacing the

vanillin reagent with 4% HCl in methanol. The standard used was catechin; tannin content was expressed as mg catechin equivalents per g (mg CE/g). Tannin content was reported with and without blank subtractions.

Sample extraction and preparation of procyanidin extracts

The method of sample extraction and procyanidin purification was adapted from Gu et al (2002). The grain, freeze dried samples and extrudates, were milled to pass through a 1 mm screen using a UDY cyclone mill (Model 3010-030, UDY Corporation, Fort Collins, CO.). 0.5 to 1 g samples were extracted using 10 mL acetone: water: acetic acid (70: 29.5: 0.5) mixture for 2 hr at low speed in an Eberbach shaker (Eberbach Corp., Ann Arbor, MI). The extracts were centrifuged, and 7.5mL supernatant recovered and evaporated to dryness at 25^{o}C in a Speed Vac SC201A (Thermo, Marietta, OH) under vacuum. The dried residue was dissolved in 6 mL water and applied to a Sephadex LH-20 column. The Sephadex column was prepared by equilibrating 3g of Sephadex LH-20, with water for over 4 hr and then manually packed into the column.

The loaded crude extract was washed with 40 mL of 30% methanol in water to remove the sugars and other low molecular weight phenols. The procyanidins were recovered from the column by using 80 mL of 70% aqueous acetone. The eluted liquid was evaporated to dryness in a Speed vac set at medium heat (43^{o}C). The dry residue was dissolved in 70% aq. acetone and made up to a final volume of 5 mL and filtered using a Whatman nylon membrane filter unit (0.45 μm) (Whatman International Ltd, Maidstone, England), before injecting into the HPLC.

HPLC analysis

The Waters HPLC system (Waters, Millford, MA) was used. It consisted of Waters 717 Plus Autosampler, Waters In-Line Degasser, Waters 600E System Controller and the Waters 474 Fluorescent detector. The system was run using the Waters Empower software.

The modified method of Gu et al (2002) was used. The mobile phase was (A) dichloromethane, (B) methanol, and (C) acetic acid/water (1:1 v/v). The gradient was 0-30 min, 14.0-28.4% B; 30-45 min, 28.4-39.6% B; 45-50 min, 39.6-86.0% B; 50-55 min,

86.0 B isocratic, 55-60 min, 86.0-14.0% B; followed by 10 min re-equilbration of the column before the next run. A constant 4% C was maintained throughout the gradient. Flow rate was 1 mL/min. Separation was on a normal-phase 5-μL Luna silica column (250 x 46 mm) (Phenomenex, Torrance, CA). Fluorescence detection was used; excitation – 276 nm, emission – 316 nm.

The normal phase HPLC method resolved procyanidins up to octamers (DP 8), based on molecular weight; thus procyanidins were reported as monomers (DP 1), oligomers (DP 2-8), and polymers (DP>8) were resolved in a single peak. Total extractable procyanidins were obtained by adding the monomer, oligomer and polymer contents.

Experimental design and data analysis

The total phenols, tannin and HPLC determinations were means of triplicate analyses. The means were analyzed with one way analysis of variance (ANOVA), and then separated using Fisher's least significant difference (LSD) at $P<0.05$. The statistical software SPSS version 11.5 (SPSS Inc. Chicago, IL) was used. Correlation was determined using Pearson's correlations.

RESULTS AND DISCUSSION

Phenols and tannin and procyanidin content of grain, porridges and extrudates

Sorghum type and processing significantly affected total phenols and procyanidin content (Table VI). The sorghums had significantly different phenolic and procyanidin contents. Processing decreased extractable phenols, tannin (vanillin-HCl method) and procyanidins (HPLC) in sorghum porridges and extrudates. Extrudates had the highest decrease in phenols assayed by these three methods. These observations confirm findings by other researchers who reported a decrease in assayable phenolic content particularly during extrusion cooking of sorghum (Taylor and Daiber 1992, Awika et al 2003a, Dlamini et al 2005).

Although the vanillin-HCl, Folin Ciocalteu and normal phase HPLC methods for determining tannin, total phenols and procyanidn contents respectively, cannot be

directly compared, generally sorghum grain and products with high total phenols and tannin content also had higher levels of extractable procyanidins. The procyanidin levels determined by HPLC were higher than the total phenols and tannin content, due to increased extractability of procyanidins in acidified aqueous acetone. For example, Framida had the highest total phenols (20.7 mg GAE/g), tannins-vanillin HCl assay (47.8 mg CE/g) and extractable procyanidin (PC) (59.1 mg/g), compared to the other grains. Early Sumac had higher PC content (36.7 mg/g) than NS 5511 (26.4 mg/g), despite its lower tannin content. The lower tannin content of Early Sumac was due to background color, probably from anthocyanins, resulting in a higher blank subtraction and lower readings. There is also a possibility that Early Sumac phenols had increased extractability in acidified aqueous acetone. Aqueous acetone is reported as more effective extractant of procyanidins than acidified methanol (Kaluza et al 1980). The addition of acetic acid further enhanced the extraction capability of aqueous acetone (Gu at al 2002).

Framida raw grains with the highest extractable procyanidin content had the highest ABTS antioxidant activity (427 μmol TE/g). However NS 5511 grain had lower procyanidin content than Early Sumac, but much higher antioxidant activity (384 μmol TE/g), while that of Early Sumac grain was 262 μmol TE/g (Chapter III). The different extraction solvents can be partly used to explain the responses of NS 5511 and Early Sumac. Acidified methanol (extractant for ABTS assay) may be a better phenol extractant for NS 5511, while acidified aqueous acetone is more efficient for extracting phenolics in Early Sumac grain.

Table VI
Total phenols, tannin content and procyanidin (HPLC) content in sorghum grain, porridge and extrudates

Sample	Total phenols	Tannin content		Total extractable Procyanidins (mg/g) (HPLC)
		WB[d]	BS[d]	
Framida grain	20.7 a	63.8 a	47.8 a	59.1 a
Framida porridge	13.4 c	23.6 e	13.7 c	26.2 c
Framida extrudates	5.3 g	4.5 h	0.4 e	11.2 e
NS 5511 grain	18.1 b	56.5 b	49.0 a	26.4 c
NS 5511 porridge	8.7 e	11.9 g	6.6 d	12.5 d
NS 5511 Extrudates	6.7 f	5.5 h	1.9 e	4.4 e
Early Sumac grain	19.4 ab	34 d	19.6 b	36.6 b
Early Sumac porridge	11.1 d	19 f	4.5 de	20.8 c
Early Sumac extrudates	11.5 d	12 g	8.6 d	14.3 d

[a]Total phenols expressed as mg gallic acid equivalents /g sample (mg GAE/g), dry weight basis (Folin-Ciocalteu method).
[b]Tannin content expressed as mg catechin equivalents/g (mg CE/g), dry basis.
[c]Procyanidin content determined using normal phase HPLC (Gu et al 2002, Awika et al 2003b)
[d]WB - without blank subtractions; BS- with blank subtractions
Framida and NS 5511 extrudates prepared using a twin-screw extruder; Early Sumac extrudates prepared using a single-screw, friction type extruder
Data within the same column with different letters are significantly different at $P<0.05$

The total phenols and tannin contents were significantly correlated to total procyanidin content determined by the HPLC method, ($r = 0.87$ and $r = 0.77$, $p < 0.01$) respectively (Table VII). Significant correlations between total phenols and tannins with ABTS antioxidant activity confirm previous reports (Awika et al 2003a, Dykes et al 2005). In addition, ABTS antioxidant activity was significantly correlated with extractable procyanidins ($r = 0.81$, $p < 0.001$). The slightly lower correlation of ABTS with procyanidin content may be due to differences in the extraction solvent. For total phenols, tannins and ABTS, the solvent was acidified methanol, while for procyanidins, the solvent was acidified aqueous acetone. These solvents have different capabilities as mentioned earlier.

Table VII
Pearson's Correlation Coefficients of total phenols, tannin and total procyanidin contents of sorghum grain and products

	ABTS	Procyanidins	Tannin content
Total phenols	0.90	0.87	0.85
Tannin content	0.95	0.77	
Procyanidins	0.81		

Correlation is significant at $p < 0.001$

Effect of sorghum type and processing on procyanidin profile

The procyanidin content and profile was influenced by sorghum type and processing method. The predominant procyanidins were polymers (DP>8), making up 68-80% of the total, followed by the oligomers (DP 2-8), 5.5-18.5 mg/g, comprising 20-31% of the total (Table VIII). The monomer (cyanidin) content was low (0.01-0.5 mg/g). Since procyanidins are reactive in the food matrix, it is important to consider the modifying influence of food processing on extractable procyanidin levels and structure.

Extrusion cooking or conventional cooking reduced total extractable procyanidins. The procyanidin polymers (DP>8) were significantly reduced by porridge and extrusion cooking in all three tannin sorghums, compared to the respective raw grains. In contrast, the levels of procyanidin oligomers in Early Sumac and NS 5511 porridges were not significantly different from that of the respective grains. Framida porridges showed a decrease in oligomer content, from 18.5 to 10.8 mg/g. However, extrusion cooking increased the extractability of monomeric procyanidins in Framida and NS 5511 extrudates.

Processing increased the proportions of oligomers (DP 2-8) and monomers except for Early Sumac extrudates, which did not change (Figs. 8, 9 and 10). The polymer proportion of Early Sumac extrudates (78%) did not differ significantly from those of the grain (80%). In extruded NS 5511 and Framida, the polymer proportion was reduced significantly, from 79 to 25% for NS 5511, and 67% to 43% for Framida). Awika et al (2003b) observed that procyanidin polymers were more easily insolubilized during processing than oligomers. The oligomers, thus remained relatively more extractable than the polymers (DP>8), whose extractability decreased as they interacted with proteins, as well as other grain components.

Table VIII
Procyanidin content of raw sorghum grain, porridge and extruded grain

DP[a]	Framida			NS 5511			Early Sumac		
	Grain	Porridge	Extrudates	Grain	Porridge	Extrudates	Grain	Porridge	Extrudates
Monomers	0.54 c	0.43 d	1.14 a	0.01 g	0.02 g	0.80 b	0.17 f	0.28 e	0.13 f
Oligomers (DP 2-8)	18.46 a	10.82 b	5.19 d	5.46 d	5.60 b	2.47 e	7.20 cd	7.67 c	2.92 e
Polymers (DP>8)	40.06 a	14.95 d	4.84 f	20.90 c	6.86 f	1.10 f	29.26 b	12.82 e	11.28 e
Total extractable procyanidins	59.06 a	26.2 c	11.17 e	26.38 c	12.48 d	4.37 e	36.63 b	20.77 c	14.34 d

[a]Framida and NS 5511 extrudates prepared using a twin-screw extruder; Early Sumac extrudates prepared using a single-screw, friction type extruder
[b]Procyanidin content (mg/g dry basis), obtained by normal phase HPLC method (Gu et al., 2002).
[c]DP-Degree of polymerization
[d]Values within the same row with different letters are significantly different at $P<0.05$.

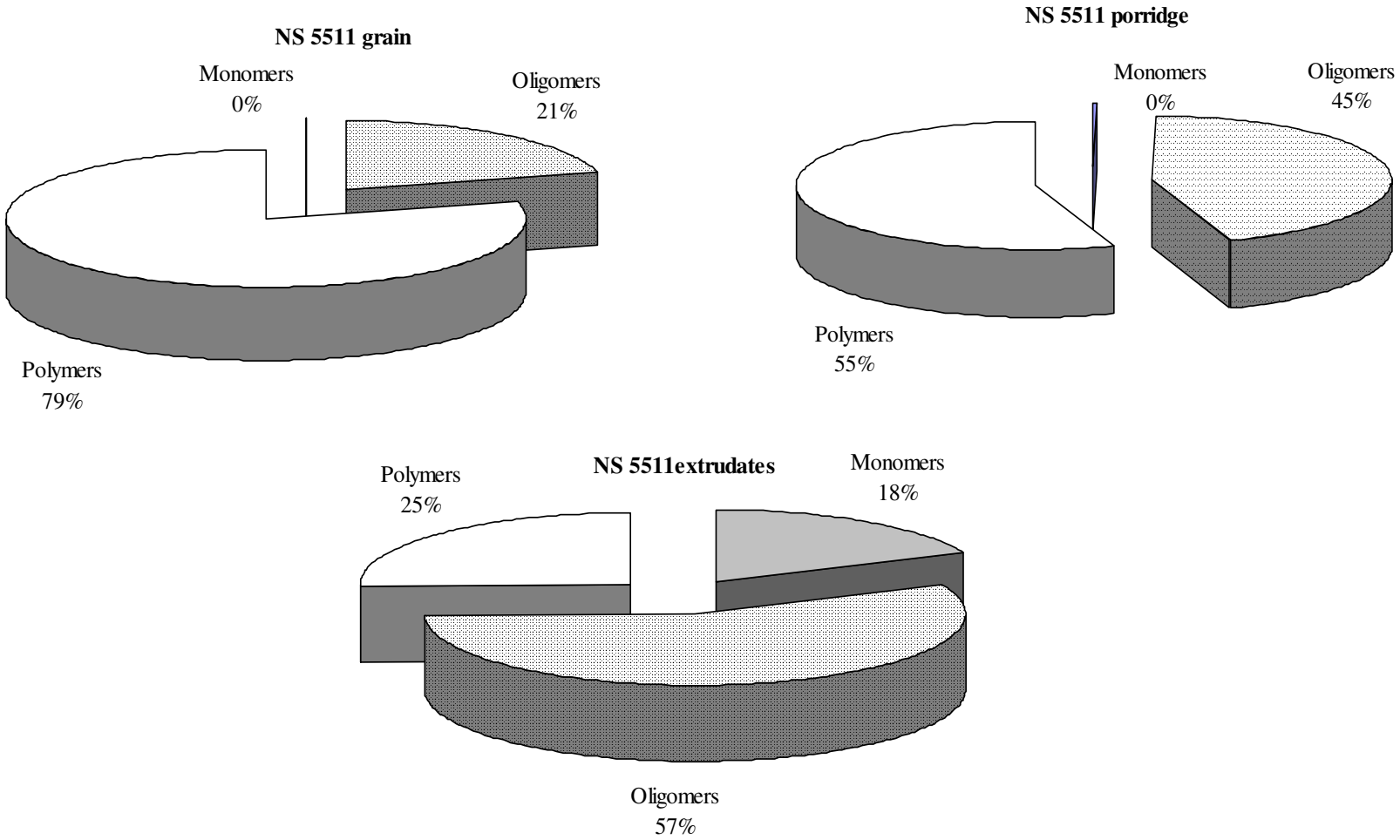

Fig. 8. Effect of processing on procyanidin monomer, oligomer and polymer distribution in NS 5511 sorghum grain, porridge and extrudates.

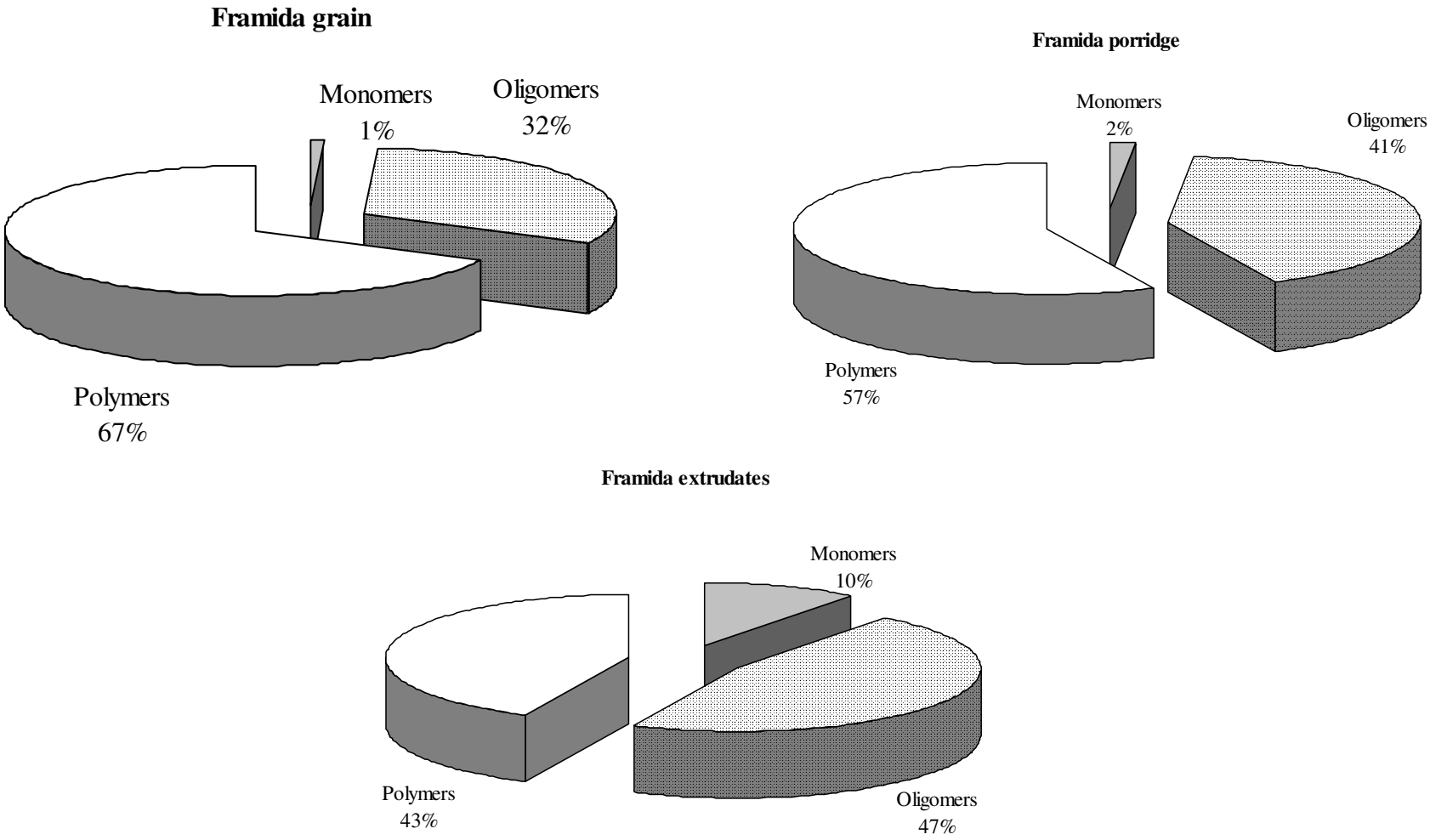

Fig. 9. Effect of processing on procyanidin monomer, oligomer and polymers distribution of Framida grain, porridges and extrudates.

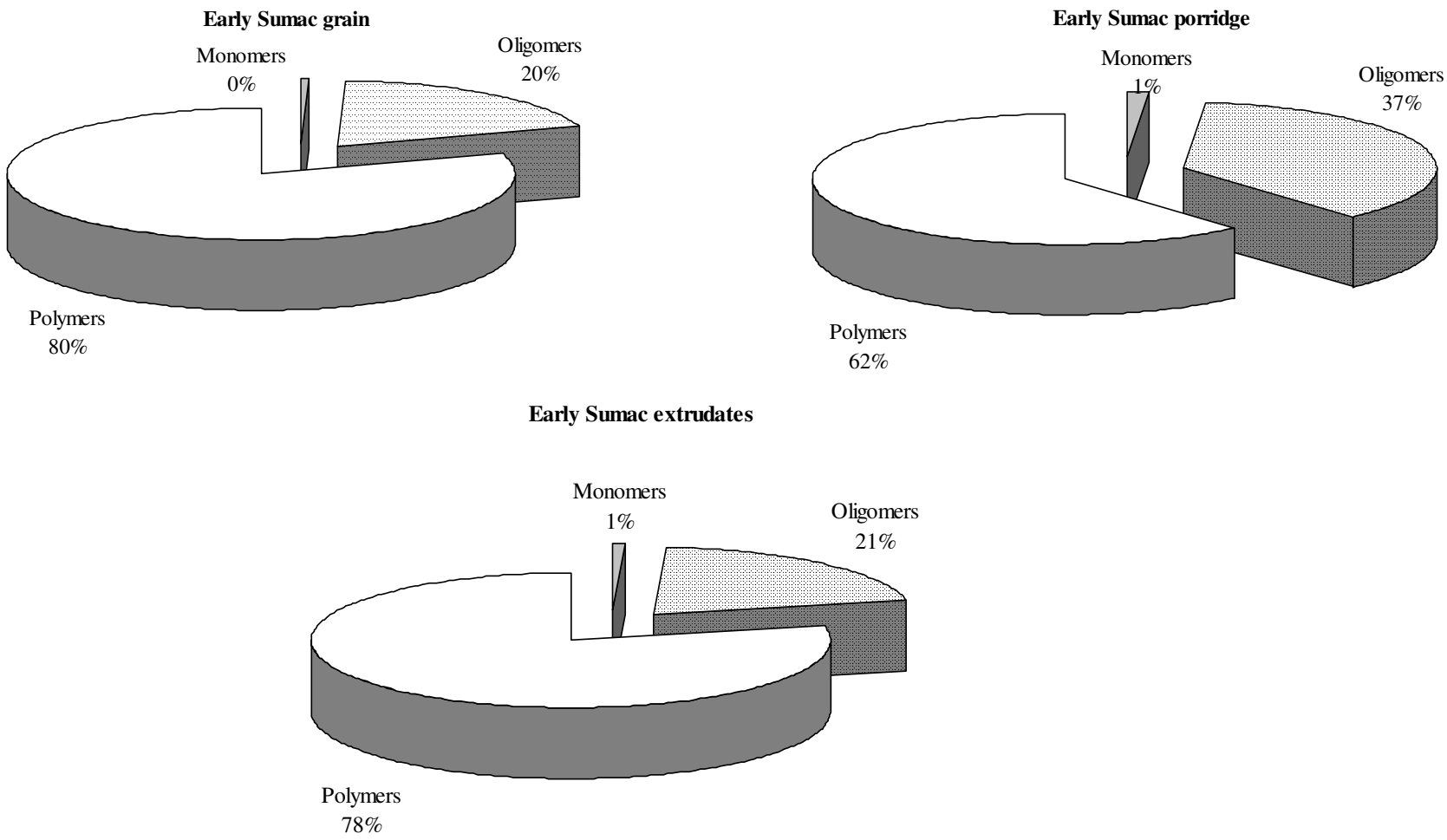

Fig. 10. Effect of processing on procyanidin monomer, oligomer and polymer distribution in Early Sumac sorghum grain, porridge and extrudates.

Effect of processing on procyanidin polymers

During processing procyanidin polymers (DP>8 or 9) interact the most protein and carbohydrates (Mehansho et al 1987), which reduces their extractability. Procyanidin oligomers (DP 2-8) in extrudates interacted more than those in porridges. Procyanidin interactions are mostly non-covalent interactions and may involve hydrogen bonding and hydrophobic interactions (Renard et al 2001). Procyanidins have strong affinity for proteins high in proline content like the prolamins (Emmambux and Taylor 2003), and strong hydrophobic associations, further stabilized by hydrogen bonding are formed (Baxter et al 1997). Hydrophobic interactions increase as molecular weight of procyanidins increases. Other hypotheses that have been proposed to explain tannin-protein interactions include π-π stacking, π-σ attraction for phenylalanine (Verge et al 2002). The hydrophobic interactions because of their stability; would probably predominate at elevated temperatures during both porridge making and extrusion cooking. When the porridge cools, hydrogen bonds are re-formed, and reinforce the hydrophobic interactions (Verge et al 2002). The stability of hydrophobic interactions may be responsible for the reduced extractability of procyanidins, even in acidic conditions.

Polyphenols may form complexes with polysaccharides probably by adsorption mediated by hydrogen bonding and hydrophobic interactions (Renard et al 2001). Hydrophobic interactions are favored by the existence of hydrophobic cavities and crevasses such as the internal cavity of cyclodextrins known to encapsulate hydrophobic compounds of appropriate shape (Renard et al 2001, de Freitas et al 2003).

Comparing the effect of porridge-making and extruder type on procyanidins

Porridges retained higher total procyanidin extractability, than the extruded grain (Fig. 11). The porridges had higher polymer retention (Fig. 12). This is an indication of different levels of procyanidin interactions within the products, the extrudates having more interactions than porridges.

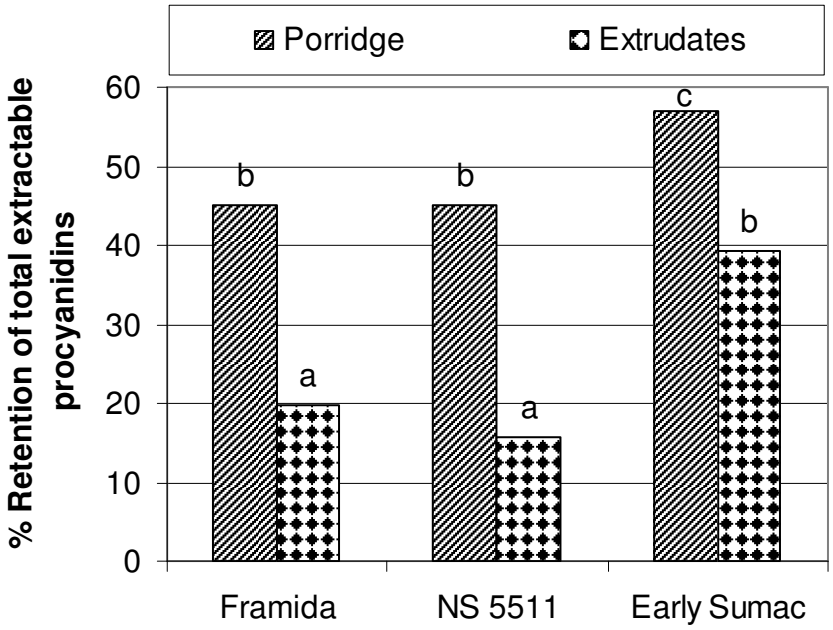

Fig. 11. Retention in extractable procyanidin oligomers (DP 2-8) in porridges and extrudates. Bars with different letters are significantly different at $p<0.05$.

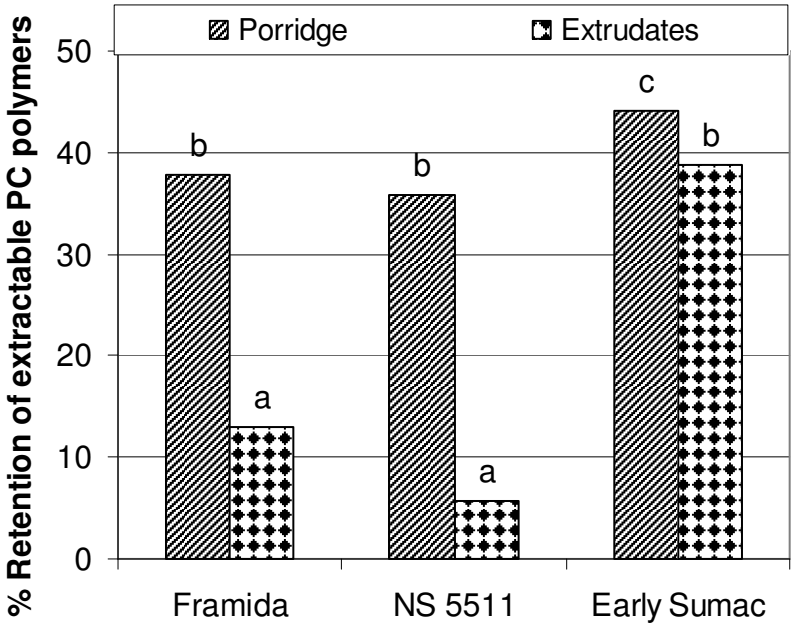

Fig. 12. Retention of extractable procyanidin polymers in porridges and extrudates. Bars with different letters are significantly different at $p<0.05$.

It is probable that high friction and shear generated during extrusion and denatures protein, and reduce protein-protein and starch-protein associations (Fellows 2000), thus the proteins can intimately interact with PC polymers, reducing their solubility. During porridge making there is low shear, and the sorghum protein can crosslink via disulphide bonds (Duodu et al 2003), thus leaving less opportunity for interaction with procyanidins.

The Early Sumac extrudates, produced using the single-screw, friction type extruder retained 39% extractable procyanidins, while NS 5511 and Framida extrudates produced on a twin screw extruder; retained much lower amounts, 17% and 19%, respectively. Although the two extrusion systems could not be directly compared because of different varieties of sorghum, the general observation was that in the single screw extruder there were less procyanidin interactions, which could have been attributed to lower moisture content of the feedstock (14%), compared to that used in the twin screw extruder (18%). In a single screw extruder lower moisture generates friction and heating up of the product. Friction also resulted in high shear, which would most likely cause molecular fragmentation, rather than interactions. In the twin screw extruder, because of higher moisture content, there was less shear, and the two rotating screws promoted mixing (van Zuilichem et al 1999), and thus more opportunities for procyanidin interaction with protein.

Procyanidin content of dough and bread containing 12% tannin sorghum bran

Wheat dough and bread containing 12% tannin sorghum bran had significantly reduced total phenols, tannin and extractable procyanidin contents, when compared to the quantities of the unprocessed tannin sorghum bran component (Table IX). Total phenols were reduced from 8.3 to 3.4 mg/g in the dough, and to 2.0 mg/g in bread. The tannins in the dough could not be detected by the vanillin-HCl assay. Total extractable procyanidins were 12.4 mg/g for the tannin sorghum bran component, and was reduced by bread making to 0.6 mg/g in dough and 0.7 mg/g in bread, representing a 95% reduction in extractable procyanidins or an extractability of 5% (Table X). The procyanidin content of the dough and bread was not significantly different, $p<0.05$.

The proportion of procyanidin polymers was 93% for the sorghum bran, 83% and 57% for the dough and bread respectively. There were low levels of monomeric procyanidins in the unprocessed bran, dough and bread, although there were slightly higher proportions of monomers in bread. The presence of slightly higher amounts of low molecular weight procyanidins (DP 1 to DP 3) in the bread compared to the dough could indicate an increase in extractability of low molecular weight procyanidins during baking or heat processing. Procyanidin solubility may increase due to depolymerization caused by high temperature, pH (carbon dioxide released from the yeast), and yeast enzyme activity. Procyanidins at elevated temperatures and low pH are reportedly unstable, and depolymerize (Porter 1992).

The reduction in total extractable phenols, tannins and procyanidins in dough and bread is an indication of insolubilization of the polyphenols. In dough and bread; procyanidin polymers probably formed stable hydrophobic interactions with the gluten net work, in addition to other non covalent interactions such as hydrogen bonding. Wheat gluten is characterized by high contents of glutamine and proline and by low amounts of amino acids with charged side chains, and thus hydrophobic interactions contribute significantly dough formation (Hoseney 1994, Weiser 2007). Hydrophobic interactions are different from other bonds, because their energy increases with increasing temperature; this can provide additional stability during baking (Weiser 2007).

There is similarity between gluten and sorghum kafirins, because both proteins have high proportions of proline, thus implying that procyanidin interactions were similar in porridges and bread. There is the issue of sorghum variety differences; a direct comparison would have been with bran from the same variety of sorghum. However, from a general observation, the bread and dough had significantly reduced extractability of procyanidins compared to porridges and extrudates. The possible explanation could be that in bread and dough, the gluten forms a viscoelastic network which may encapsulate the procyanidins, thus promoting more intimate interactions, or the gluten proteins are more hydrophobic.

Table IX
Total phenols and tannin content in bread mix, dough and bread with 12% tannin sorghum bran

Sample	Total phenols[a]	Tannin content[b]		Total extractable procyanidins[c] (normal phase HPLC)
		WB	BS	
Tannin sorghum bran component (bread mix)	8.3 a	31.0	22.9	12.4 a
Dough containing 12% tannin sorghum bran	3.4 b	ND	ND	0.6 b
Bread containing 12% tannin sorghum bran	2.0 c	ND	ND	0.7 b

[a]Total phenols expressed as mg gallic acid equivalents /g sample, dry weight basis (Folin-Ciocalteu method).
[b]Tannin content expressed as catechin equivalents/g, dry basis. WB - without blank subtractions, BS- with blank subtractions
[c]Procyanidin content (mg/g dry basis), obtained by normal phase HPLC method (Gu et al2002).
[d]ND- not detected
[e]Data within the same column with different letters are significantly different at $P<0.05$

Table X
Procyanidin content of bread mix, dough and bread containing 12% tannin sorghum bran

DP (degree of polymerization)	Tannin sorghum bran component (bread mix)	Dough (12% tannin bran)	Bread (12% tannin bran)
Monomers	0.01 b	Not detected	0.01 a
Oligomers (DP 2-8)	1.0 b	0.2 a	0.3 a
Polymers (DP>8)	11.5 a	0.5 a	0.4 a
Total extractable procyanidins	12.4 a	0.6 a	0.7 a

[a]Procyanidin content (mg/g) obtained by normal phase HPLC, method of Gu et al (2002)
[b]DP-Degree of polymerization
[c]Mixture of polymers with DP>8
[d]Oligomers (DP 2-8), in mg/g, dry basis, and in parenthesis expressed as a per cent total
Values within the same column with different letters are significantly different at $P<0.05$

Sorghum procyanidin distribution and its implications on astringency and bioavailability of procyanidins

Procyanidin polymer content is associated with astringency. The sensation of astringency is associated with the interaction of salivary proteins and procyanidin polymers (DP>8); processing conditions that affect extractability of these polymers affects astringency. The significant reduction in polymers for the NS 5511 extrudates when compared to porridges should be associated with a reduction in astringency of the product. Other factors like carbohydrates and other ions that interfere with protein/polyphenol interactions, and subsequent aggregation affect astringency (de Freitas et al 2003). The presence of water-soluble polysaccharide fragments, such as pectin fragments lead to decreased astringency in wines. The encapsulation of procyanidins by gel structures ie xanthan gums may decrease the interaction of procyanidins with salivary proteins, thus decreasing astringency (de Freitas et al 2003).

The bread making process should reduce astringency of tannins from the bran. The procyanidins may be encapsulated by the gluten network, which prevents their interaction with salivary proteins. The phenomena might be similar to that observed for polysaccharides such as xanthan and gum Arabic, where the gel, encapsulates the polyphenols (de Freitas et al 2003). The presence of sodium chloride ions, products from yeast fermentation and sugars affect the perception of astringency. Depolymerization due to yeast and other microbial activities is also a possibility since the bread is processed for two hours, and then baked for 50 minutes, a total processing time of 2 hours 50 minutes.

It is generally accepted that PC oligomers are more bio-available than PC polymers. An *in vitro* study showed that PCs up to trimers were absorbed through intestinal cell mono layers (Deprez et al 2000). Spencer et al (2000) demonstrated that oligomers DP<6 were depolymerized to monomers in acid or simulated gastric juice environment. Rechner et al (2002) showed that procyanidins were cleaved colonic bacterial enzymes and subsequently absorbed and metabolized in the liver into

glucuronides of 3-hydroxyphenylacetic acid, homovanillic acid, vanillic acid and isoferulic acid.

Bawadi et al (2005) showed that the growth of colon, breast and prostatic cancer cells was inhibited by black bean procyanidins, which were comprised of mainly trimers and tetramers. Faria et al (2006) observed a decrease in peroxyl free radical scavenging ability of more complex procyanidins, and explained that the observation could be due to possible steric hindrance, which limits the access of the ortho-catechol groups. Our findings showed a strong correlation of ABTS antioxidant activity with procyanidin polymers indicating that most of the antioxidant activity was from the polymers.

On the whole, processing appeared to increase the levels of cyanidins, and decreased the higher molecular weight procyanidins (DP>8)**.** The increase in extractability of low molecular weight procyanidns indicates that there might be an increase in bioavailability, and thus potential health benefits. The hydrolysis of the food matrix with amylase and proteases might free entrapped procyanidin molecules. Further studies are required on the fate of the unextractable procyanidins once they get to the gut.

CHAPTER V

EFFECT OF ENZYME HYDROLYSIS ON RECOVERY OF PHENOLS AND ANTIOXIDANT ACTIVITY IN SORGHUM AND PROCESSED PRODUCTS

The goals of the study were to determine the effect of *in vitro* enzymatic hydrolysis on the recovery of phenols and antioxidants from processed sorghum products. Processing of tannin sorghums significantly reduced extractability of phenols including condensed tannins, leading to reduced antioxidant activity. The method of Goni et al (1997); which used pepsin followed by amylase hydrolysis of foods to measure estimated glycemic indices was used to hydrolyze the processed sorghum foods. In addition, the samples were hydrolyzed by pepsin and amylase alone to determine how starch and protein affected extraction of the antioxidants. Pepsin alone and pepsin followed by amylase hydrolysis significantly increased antioxidant activity of porridges and extrudates prepared from whole grain tannin and non-tannin sorghums. The tannin sorghum products had higher antioxidant activity than the non-tannin products. However, amylase alone did not significantly improve the recovery of antioxidant activity which suggested that the starch chains are not too important in reducing the extractability of antioxidants in the processed grains. The high antioxidant activity of pepsin hydrolyzed non-tannin sorghum products is an indication that peptides containing aromatic amino acids play a significant role in increased antioxidant activity. It is not clear if this effect is due to release of tyrosine or other amino acid residues. In addition to peptides, tannin content increased in the supernatants of pepsin treated samples compared to those from amylase or control treatments, as indicated by tannin content of the residues. Hydrolysis of food by pepsin and the acids in the stomach could enhance antioxidant activity in the stomach and small intestine; thus, protecting the alimentary system from free radicals generated during digestion. Pepsin definitely increases tannin extractability from processed samples; additional studies are required to identify the specific antioxidants released during pepsin hydrolysis.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] varieties that contain condensed tannins have the highest level of antioxidants of any cereals analyzed (Gu et al 2004), and thus are recognized as important sources of dietary antioxidants (Awika and Rooney 2004, Dykes and Rooney 2006). Measurable phenolic compounds, including tannins and total phenols in unprocessed sorghum grain were strongly correlated with antioxidant activity measured by the ABTS, DPPH and ORAC methods (Awika et al 2003a, Dykes et al 2005). The processing of sorghum by fermentation (Hassan and El Tinay 1995, Bvochora et al 1999), extrusion cooking (Taylor and Daiber 1992) and porridge cooking (Dlamini et al., 2005) reduces measurable tannins, which lowers *in vitro* antioxidant activity of the processed foods.

Tannins interact with proteins and carbohydrates (Mehansho et al 1987), which reduces their extractability. Tannin interactions with food components are mostly non-covalent interactions, and may involve hydrogen bonding and hydrophobic interactions (Asquith and Butler, 1986; Mehansho et al 1987, Renard et al 2001). Sorghum tannins have strong affinity for proteins high in proline content like the prolamins (Emmambux and Taylor 2003). The tannins form strong hydrophobic associations with proline-rich peptide fragments; these associations are further stabilized by hydrogen bonding (Baxter et al 1997). There is evidence that tannins may associate with other grain pigments, such as anthocyanins (Remy et al 2000); these complexes are probably not detected by the vanillin method for tannins.

There are numerous reports on maximizing tannin extraction from raw unprocessed leaves, fruits and grains, but there has been limited information on extractions from processed samples. The usual assumption is that during processing tannins are either destroyed or structurally modified. Tannin structure modification occurs under extreme temperatures and pH. Condensed tannins undergo polymerization at alkaline pH to form higher molecular weight polymers that are highly cross-linked and insoluble (Porter 1992). Work done by Beta et al (2000) showed that prior treatment of

tannin sorghum with alkaline solutions reduced assayable tannins and improved malt quality. Under heat and acidic conditions tannins depolymerize to oligomers and monomers, the basic phenolic structure remaining stable (Porter 1992). Most food processing conditions do not have these extreme conditions, so these side reactions of tannins are probably minimal.

It is theoretically possible that tannin-protein complexes in food can be dissociated and tannins recovered. Disrupting the food matrix, especially starch, may free tannins that are entrapped as the processed food such as porridges, starch gelatinizes and retrogrades, and as setting takes place in bread. Thus amylases should have an effect since sorghum products contain more than 70% starch. Destabilization of tannin-protein complexes, for example, by protein hydrolysis should be effective. Using pepsin treatment, Ferruzi and Green (2006) observed an increase in recovery of tea catechins from dairy matrices. However, there are suggestions that tannin-protein complexes are less digestible, probably due to the hydrophobic nature of the association that limits enzyme penetration (Duodu et al 2003). Rao et al (1984) reported an increase in tannin extraction from de-oiled seed cakes that were treated with low frequency (43 kHz) ultra sound irradiation.

The main objective of the study was to evaluate the effect of amylase and pepsin hydrolysis of sorghum extrudates and porridges on phenol extractability and antioxidant activity. Phenol extractability was assessed, using the Folin Ciocalteu assay, both in the supernatant and in the residues remaining after enzyme digestion. The Goni et al (1997) method for estimating glycemic index of foods was used. Thus the study simulated or attempted to mimic digestion. This study may be important in the development of *in vitro* digestion methods to assess bioavailability of antioxidants from meals. *In vivo* methods are complicated, expensive, and may involve ethical issues.

MATERIALS AND METHODS

Materials

Three tannin type sorghums were used; NS 5511 and Framida were grown in 2003 in Zimbabwe and Early Sumac was grown in College Station, Texas, USA in 2003. The white, non tannin sorghum; Macia was from Zimbabwe and grown in 2003.

Tannin sorghum bran was obtained by decorticating tannin sorghum grain (ATx 623 x RTx SC103 (College Station, Tx. 2003) and white non-tannin sorghum (ATx 635 x RTx 436) in 4-kg batches in a PRL mini-dehuller (Nutama Machine Co., Saskatoon, Canada). The bran was separated with a KICE grain cleaner (Model 6DT4-1, KICE Industries Inc., Wichita, KS). The bran (approx. 18% of the original grain weight) was further milled into a fine powder.

Catechin hydrate, gallic acid, potassium persulfate, and the enzymes α-amylase from porcine pancreas and pepsin from porcine stomach, were obtained from Sigma (St Louis, MO). The Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid) was obtained from Acros Organics (Morris Plains, NJ), while 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from TCI Kasei Koygo (Tokyo, Japan).

Preparation of the sorghum porridges

The whole grains Macia, Ns 5511, Framida and Early Sumac were milled through a 1 mm screen using a coffee grinder and then prepared into porridges. The milled grain (78.3 g) was added to 100 mL cold water; the slurry was added to 333 mL of boiling water and cooked with constant stirring for 10 min. The porridge was put into plates, cooled for about 30-60 min, and frozen using liquid nitrogen and freeze-dried. The freeze-dried samples were stored at -20^{o}C until analysis.

Extrusion cooking of sorghum

The NS 5511 and Framida whole sorghum grains were coarsely milled to screen size 1.58 mm using a Hippo hammer mill (Precision Grinders Engineers, Harare, Zimbabwe), and then extruded in a Clextral BC92 twin-screw, co-rotating extruder (Clextral, FIRMINY Cedex, France). The feed rate was 550 kg / hr; moisture content of

feed was adjusted to 18%, by injecting water at 45 L / hr. The screw rotation speed was 230 rpm, and barrel temperature was maintained at 150 and 160^{o}C, residence time was 30-90 sec. The die diameter was 2 mm and the cutter speed set at 120 rpm. After extrusion, the sorghum extrudates were placed in containers and allowed to cool and equilibrate for a few hours (4-5 hr). The final moisture content was 6-8%.

The Early Sumac extrudates were prepared according to the method described by Perez, (2005). The whole grains were tempered to 14% moisture, and then cracked into ½-1/8 pieces of grain in an attrition mill (Glenn Mills Inc., Maywood, NJ). The cracked grain was extruded using a single-screw friction-type extruder (Model MX-3001, Maddox Manufucturing Co, Inc, Dallas, Tx). The extruder was preheated to 150^{o}C; the product was cooked by heat generated by friction. The feed rate was 158.8 kg/hr, and screw rotation speed was 341 rpm. The die diameter was 3.18 mm. After extrusion the extrudates were dried at 100^{o}C for 30 min; final moisture content was 3.5%.

Preparation of bread containing tannin and non tannin sorghum bran

Wheat bread containing 12% tannin or white non-tannin sorghum bran was prepared using refined wheat flour (Gold Medal Superlative, General Mills Inc., Minneapolis, MN), using a modified method of Gordon (2001). The dough and bread were prepared in a bread machine TR800 Breadman Plus automatic bread maker (Salton/MAXIM Housewares Inc., Mt Prospect, IL). The amount of wheat flour used was 459.1 g and the setting on the bread machine was for a 680 g loaf of bread. The formula is located in Table XI. The total preparation time for the bread (kneading, proofing and baking) was 3 hr (180 min); the baking time was 50 min. After cooling, the bread was sliced and frozen in liquid nitrogen, and freeze-dried.

Table XI

Formulation of bread containing 12% tannin or white non tannin sorghum bran

Ingredient	Amount (g)	Bakers %
Enriched wheat flour	459.1	100
Water	323.4	62.0
Sorghum bran	62.6	12.0
Sugar	31.3	6.0
Salt	7.8	1.5
Active dry yeast	3.9	0.8
Vital wheat gluten	5.2	1.0
Sodium stearoyl lactylate	1.6	0.3
Oil	13.8	3.0

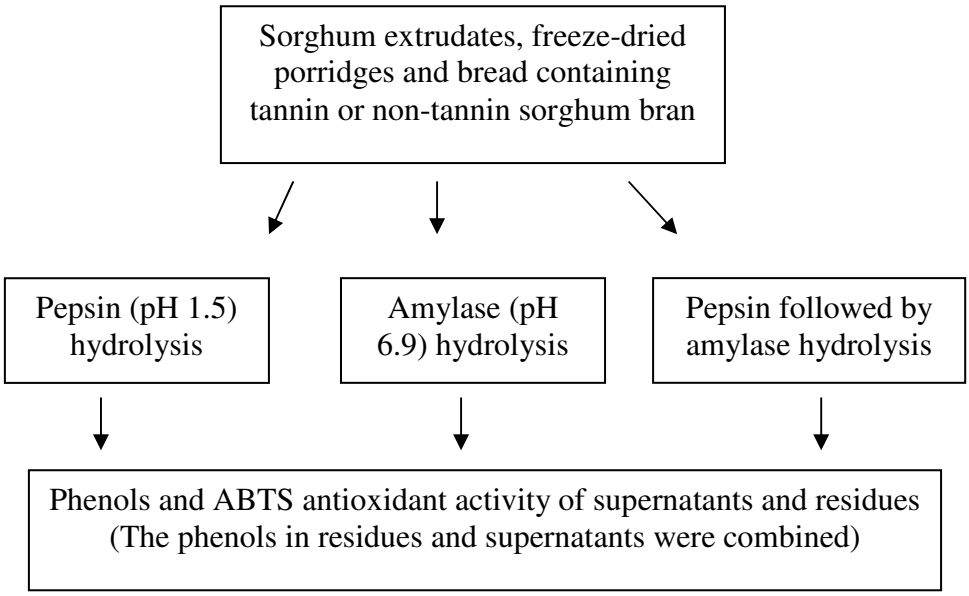

Fig. 13. Experimental design of amylase, pepsin and pepsin followed by amylase hydrolysis of sorghum extrudates, porridges and bread containing 12% tannin or white, non-tannin sorghum bran. The supernatants and residues from enzyme treatments were analyzed.

Enzyme hydrolysis of sorghum products

The enzyme extrudates, freeze dried porridges and bread were hydrolyzed using amylase, pepsin and pepsin followed by amylase using the method of Goni et al (1997). The experimental design is outlined in Fig. 13.

Amylase hydrolysis

The method was adapted from Perez et al (2005). The extrudates were milled to pass through a 1 mm screen using the UDY cyclone mill (Model 3010-030, UDY Corporation, Fort Collins, CO.). Samples weighing 250 mg were incubated for 4 hr at room temperature, in 25 mL of 0.1M Tris-maleate buffer (pH 6.9) solution containing 10 units of α-amylase from porcine pancreas. The Tris-maleate buffer consisted of 0.1M Trizma salt and 4 mM $CaCl_2$ in distilled water. Enzyme blanks consisted of the ground samples incubated in Tris-maleate buffer solution only [Buffer (A)], no enzyme was added. The samples were incubated with constant shaking. The hydrolysate was centrifuged and the supernatant retained, while the residue was dried at room temperature.

Pepsin hydrolysis

The method was adapted from the Goni et al (1997) method for estimating glycemic index in food. Pepsin from porcine stomach was prepared in KCl-HCl buffer (pH 1.5), to give a solution of 1 g pepsin per 10ml; 1 mL of this solution was added to 250 mg ground extrudates. The volume was made up to 25 mL with pH 1.5 buffer and the suspensions were incubated, with constant shaking, at 40^{o}C for 2 hr. The KCl-HCl buffer consisted of mixture of 0.2M KCl (25 mL) and 0.2M HCl (16.2 mL) diluted to 100 mL using distilled water. The enzyme blanks consisted of samples incubated in KCl-HCl buffer (pH 1.5) only [Buffer (P)]. The suspensions were centrifuged, the supernatant retained and the residue air-dried at room temperature.

Pepsin hydrolysis followed by α-amylase hydrolysis

The modified method of Goni et al (1997) was used. Ground samples weighing 250 mg were suspended in 1 mL pH 1.5 KCl-HCl buffer, and 1 mL suspension containing 1g pepsin per 10 ml KCl-HCL buffer was added. The mixture was incubated

at 40^{o}C for 2 hr, after which pH was adjusted to pH 6.9 using 27 mL Tris-maleate buffer, and then Tris-buffer containing 10 units α-amylase was added and samples were incubated at 37^{o}C for 3 hr. Total volume was 30 mL. The hydrolyzed samples were centrifuged, supernatant retained, and residue air dried. The enzyme controls consisted of samples incubated in pepsin buffer (P) and then amylase buffer (A) [Buffer (P+A)].

Chemical analyses

The unprocessed grain, extrudates and freeze dried porridges were milled to pass through a 1 mm screen using UDY cyclone mill (Model 3010-030, UDY Corporation, Fort Collins, Co.). The milled samples were extracted for 2 hours using acidified methanol (1 % HCl in methanol), and constant shaking was at slow speed on an Eberbach shaker (Eberbach Corp., Ann Arbor. MI). Total phenols and antioxidant activity were determined on the clarified extract.

The dried residues from the amylase and pepsin treatments were also milled and extracted with acidified methanol and analyzed for phenols and antioxidant activity. Dry matter loss due to the solubilizing action of the enzymes was calculated, and used to obtain the percentage of phenol and antioxidant recovery. The supernatants from enzyme hydrolysis were also analyzed; phenol and antioxidant activity was expressed on dry weight of sample that was originally hydrolyzed.

The phenols and antioxidant activity from the residues and supernatants were combined in determining total phenols and antioxidant activity respectively.

Total phenols and tannin content

Total phenols were determined using the modified Folin Ciocalteu method (Kaluza et al 1980). An aliquot of the acidified methanol extract (0.1 mL) was diluted with 1.1 mL water, and then reacted with 0.4 mL Folin Ciocalteu reagent and 0.9 mL of 0.5 M ethanolamine. The reaction was carried out for 20 minutes at room temperature and absorbance was read at 600 nm. Tannin content, with blank subtractions, was determined using the modified vanillin-HCl method (Price et al 1978). The standard used for total phenols and tannins was catechin.

Antioxidant activity

Antioxidant activity was determined using the ABTS method described by Awika et al (2003a) and Re et al (1999). The standard used was Trolox and antioxidant activity was expressed as μmol Trolox Equivalent antioxidant activity per gram sample (μmol TE/g).

Formula for recovery of phenols and antioxidant activity from residue:

Amount in residue = Concentration in Residue x dry weight retained (%)

$$\text{Recovery (\%)} = \frac{\text{Amount in residue}}{\text{Amount in reference (grain)}} \times 100$$

Effects due to buffer or enzyme treatments

The effects due to enzyme hydrolysis were obtained by subtracting quantities determined in buffer from those of enzyme treatments (enzyme in buffer).

Statistical Analyses

The experiments were done in triplicate. The analysis of variance (ANOVA) was determined using the statistical package SPSS version 11.5 (SPSS Inc. Chicago, IL), and the means were separated using Fisher's least significant difference (LSD) at $P<0.05$.

RESULTS

Enzyme hydrolysis and dry matter loss of sorghum extrudates and porridges

Dry matter loss was significantly affected by food processing method and enzyme treatment (Table XII). The overall cultivar effect was not significant; although Early Sumac had slightly lower dry matter loss than the other cultivars.

The enzyme treated extrudates had higher dry matter loss (DML) than similarly treated porridges. The amylase or pepsin followed by amylase treated products had higher DML than those treated with pepsin only. This was expected since starch comprises over 70% of sorghum grain dry matter, while protein content is on average 10% (Waniska and Rooney 2001). The pepsin hydrolyzed NS 5511 grain and porridges had significantly lower DML than similarly treated Macia grain and porridge (Fig. 14). Contrary, amylase treated NS 5511 porridges had higher DML than amylase treated Macia porridges, and one of the probable explanations is the differences in endosperm

texture, where Macia had a corneous or vitreous endosperm, while that of NS 5511 was floury. Starch digestion was significantly higher in floury sorghum endosperm than vitreous endosperm (Ezeogu et al 2005).

Although the DML of pepsin treated extrudates or porridges was low there was significant increased solubility of phenols compared to untreated extrudates, thus improved antioxidant recovery.

Although the extrusion processes cannot be compared because of cultivar effects, generally amylase-treated extrudates produced in the twin screw extruder (Macia, NS 5511 and Framida), had higher DML than Early Sumac extrudates from the single screw extruder.

Table XII
Dry matter loss (%) in enzyme treated extrudates and porridges

Sample/Treatment	Macia[b]	NS 5511[b]	Framida[b]	Early Suma[c]	**Mean enzyme effect**
Extrudate-Pepsin	44.9 d	49.8 d	42.4 d	25.4 f	**40.6 C**
Extrudate- Buffer (P)[c]	40.7 d	47.3 d	45.9 d	27.4 f	**40.3 C**
Extrudate-α-amylase	63.7 b	71.5 a	70.8 a	56.8 c	**65.7 A**
Extrudate-Buffer (A)[d]	25.8 f	33.9 e	31.0 e	9.5 h	**25.1 D**
Extrudate-Pepsin+Amylase	66.2 b	71.1 a	70.6 a	66.4 b	**68.6 A**
Extrudate –Buffer (P+A)[e]	26.5 f	13.9 g	19.6 g	0mn	**15.0 D**
Porridge-Pepsin+Amylase	65.2 b	62.2 b	60.8 c	54.0 c	**60.5 B**
Porridge–Buffer (P+A)	3.9 i	0 i	0 i	0 i	**1.0 E**
Mean cultivar effect	**42.1 F**	**43.7 F**	**42.6 F**	**29.9 F**	

[a]Values with the same letter are not significantly different at P<0.05. Upper case letters are used for mean cultivar and enzyme effects.
[b]Macia, Framida, NS 5511 extrudates were produced on a twin-screw extruder, while Early Sumac was produced using a single screw friction extruder.
[c]Buffer (P)-Samples incubated in pepsin buffer, KCl-HCl buffer, pH 1.5,[d] Buffer (A)- Samples incubated in amylase buffer, Tris-maleate buffer, pH 6.9
[e]Buffer (P+A)-Samples incubated in pepsin, followed by amylase buffers.

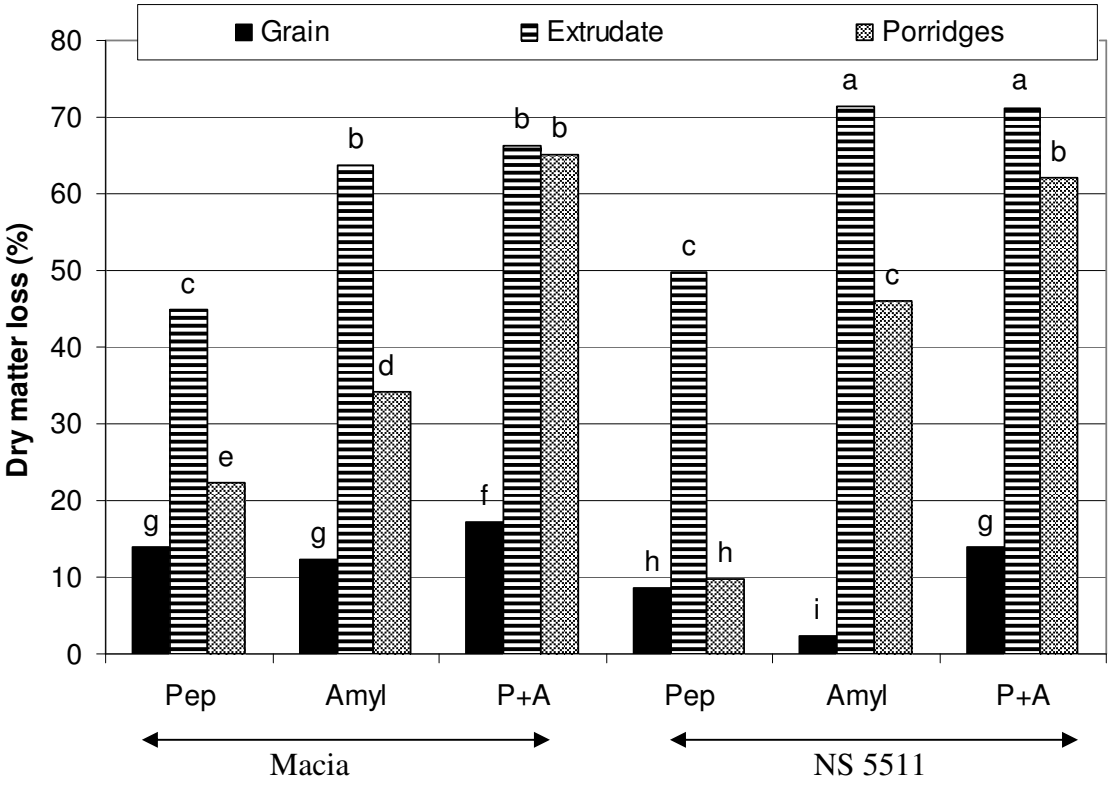

Fig. 14. Effect of sorghum type, processing and enzyme treatment on dry matter loss. Abbreviations: Pep-pepsin; Amyl-amylase; P+A –pepsin, followed by amylase.

Effect of sorghum type and processing on phenol content of sorghum products

The measurable phenols were significantly affected by sorghum type, processing and enzyme treatment (Table XIII). The tannin sorghum (NS 5511, Framida and Early Sumac) products had the highest decrease in phenols, compared to non-tannin Macia products (Fig. 15). Tannin sorghum products also had highest reductions in phenols compared to Macia. Generally the extrudates had lower phenol content than porridges, except for Early Sumac and Macia, where the phenol contents of extrudates and porridges were not significantly different ($p<0.05$).

In extruded tannin sorghums, the phenols decreased from an average 21.9 to 11.5 mg CE/g. The NS 5511 and Framida extrudates, produced on a twin-screw extruder, had significantly lower phenol recovery (22-30%) than Early Sumac extrudates (55%

recovery), produced using a single-screw friction-type extruder (Fig. 16). Framida porridge had the highest phenol levels (14.4 mg CE/g), followed by Early Sumac (11.2 mg/g), representing 59 and 60% recoveries respectively. NS 5511 porridge had lower phenol recovery (39%, 8.7 mg CE/g). As expected, Macia porridges had lowest phenol content (2.0 mg/g), but a higher recovery of 74%, while extrudates had lower phenol recovery of 67%.

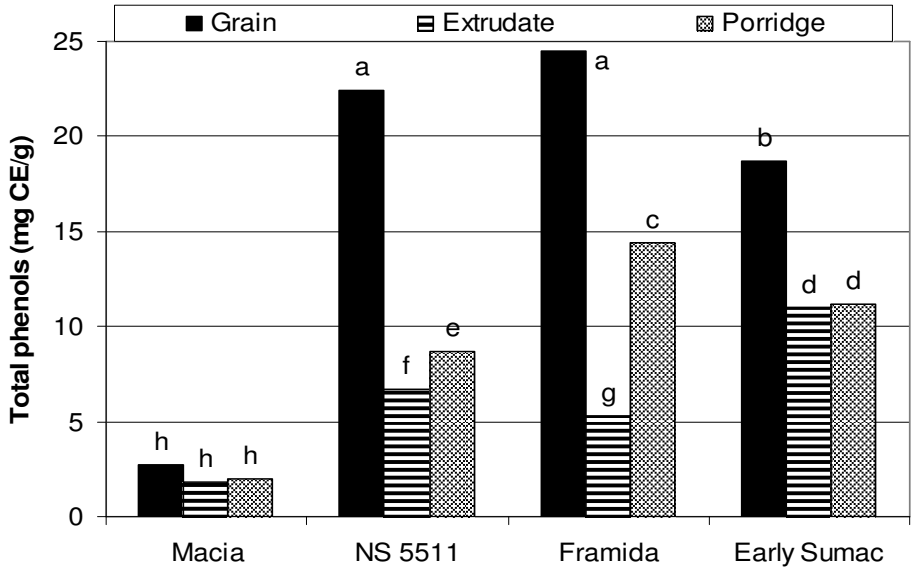

Fig. 15. Effect of processing on total phenols of sorghum extrudates and porridges, compared to the grain**.** Total phenols expressed as mg CE/g, dry basis. Bars with different letters are significantly different, $p<0.05$.

Table XIII
Effect of pepsin and amylase treatments on total phenols in sorghum grain, extrudates and porridges

Sample	Macia	NS 5511	Framida	Early Sumac	**Mean[c]**
Grain	2.7 e	22.4 a	24.5 a	18.7 a	**17.1 A**
Extrudate	1.8 e	6.7 h	5.3 f	11.0 b	**6.2 B**
Porridge	2.0 e	8.7 d	14.4 c	11.2 b	**9.1 B**
Grain-Pepsin	7.7 b	17.4 b	-	-	
Grain-Amylase	4.0 d	13.5 e	-	-	
Grain-Pep+Amyl	7.5 b	15.4 c	-	-	
Extrudate-Pepsin	7.3 bc	11.7 f	12.2 d	10.2 c	**10.4 B**
Extrudate-Amyl	4.6 d	10.0 g	10.1 e	11.4 b	**9.0 B**
Extrudate Pep+Amyl	9.4 a	14.4 d	15.7 c	18.3 a	**14.5 A**
Porr-Pepsin	5.1 d	9.8 g	-	-	
Porr-Amyl	3.1 e	11.5 f	-	-	
Porr-Pep+Amyl	6.8 c	15.6 c	20.5 b	18.2 a	**15.3 A**
Mean[b]	**4.9 B**	**12.8 A**	**14.7 A**	**14.1 A**	

Values within the same column with different letters are significantly different, $p<0.05$.
[a]Phenols expressed as mg catechin equivalents/g (mg CE/g), dry weight basis (Folin-Ciocalteu method). Total phenols in residues and supernatants.
[b]Values within the Mean cultivar effect row with different uppercase letters are significantly different, $p<0.05$.
[c]Values within the Mean enzyme effect column with different uppercase letters are significantly different, $p<0.05$
Mean values exclude enzyme treated grain, Macia and NS 5511 pepsin and amylase treated porridges.
Abbreviations: Porr – porridge; Pep-Pespin; Amyl- amylase; Pep+Amyl-Pepsin followed by amylase treatment
- No data value

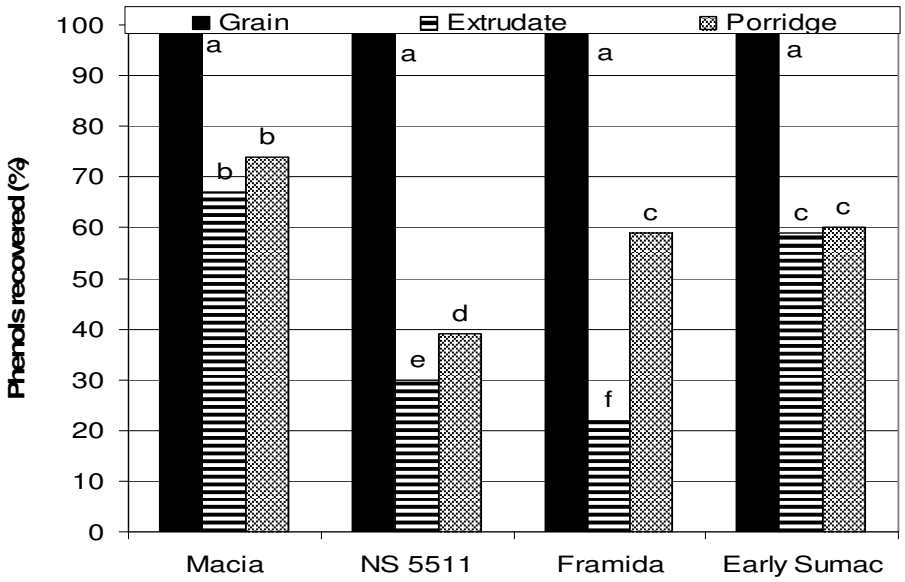

Fig. 16. Effect of processing on the recovery of total phenols in sorghum extrudates and porridges. Grain used as the reference (100% recovery). Bars with different letters are significantly different, $P<0.05$.

Effect of sorghum type and enzyme treatment of grains on phenols

The enzyme treatment of NS 5511 unprocessed grain significantly decreased phenols, while in enzyme treated Macia grain the phenols increased (Fig. 17). The pepsin or pepsin followed by amylase treatments significantly increased phenols in Macia grain almost four fold. The lower enzyme effects and the decrease in phenols on enzyme treatment of NS 5511 grain could probably indicate enzyme inhibition, probably due to tannins binding the enzymes. The proteins bind to tannins forming undigestible complexes (Papadopoulou and Frazier 2004). The effects due to α-amylase were low for both Macia and NS 5511 grains, most likely due to poor hydrolysis of native starch.

Pepsin and pepsin followed by amylase treated Macia grain had phenol recovery that ranged from 277 to 285%, while amylase treated samples had 148% recovery (Fig. 18). Enzyme treatment of NS 5511 grain reduced overall phenol recovery, with amylase treated grain having the lowest recovery.

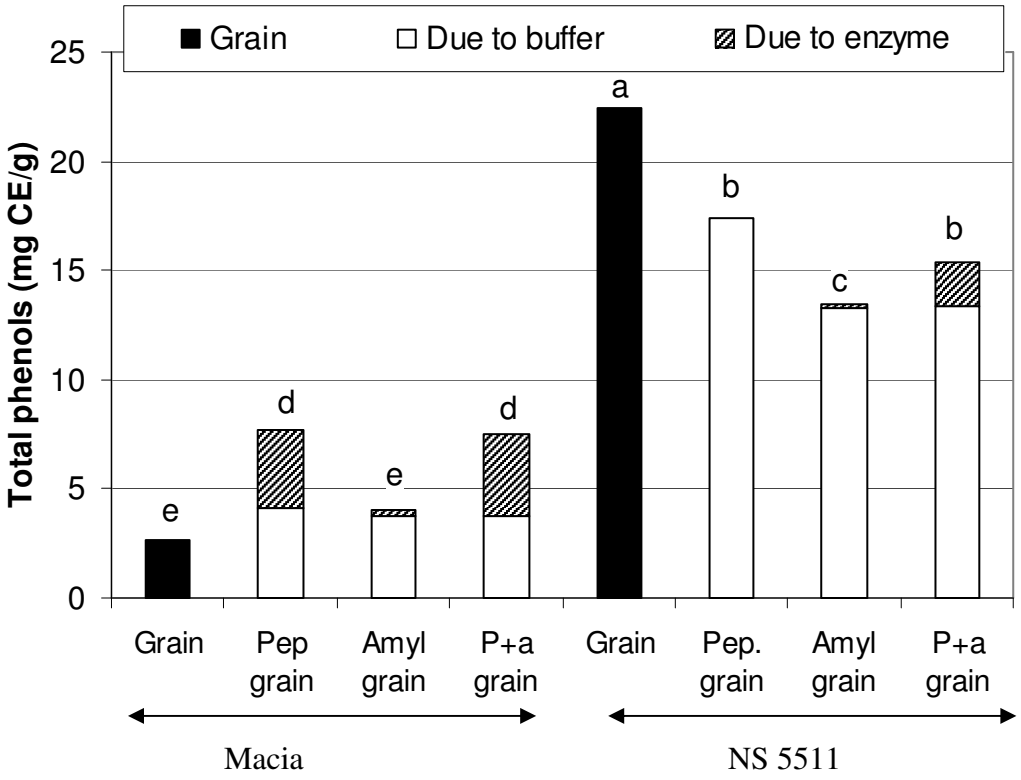

Fig. 17. Effect of enzyme hydrolysis on total phenols in Macia and NS 5511 grain. Total phenols expressed as mg catechin equivalents/g (mg CE/g), dry basis. Bars with different letters are significantly different, $p<0.05$.

Abbreviations: Pep-pepsin treated; Amyl-amylase treated; P+A-pepsin, followed by amylase treated.

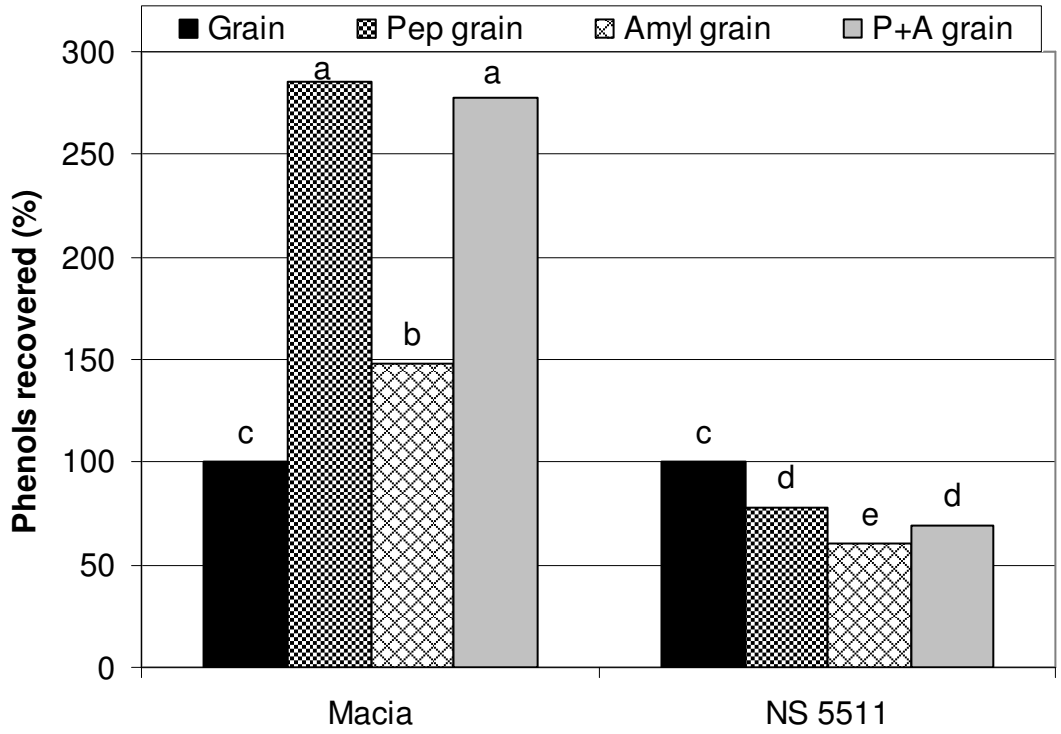

Fig. 18. Effect of enzyme hydrolysis on total phenols recovered in enzyme treated Macia and NS 5511 grain. The grain is the reference, 100% recovery. Bars with different letters are significantly different, $p<0.05$.

Abbreviations: Pep-pepsin; Amyl-amylase, P+A-pepsin, followed by amylase treatment.

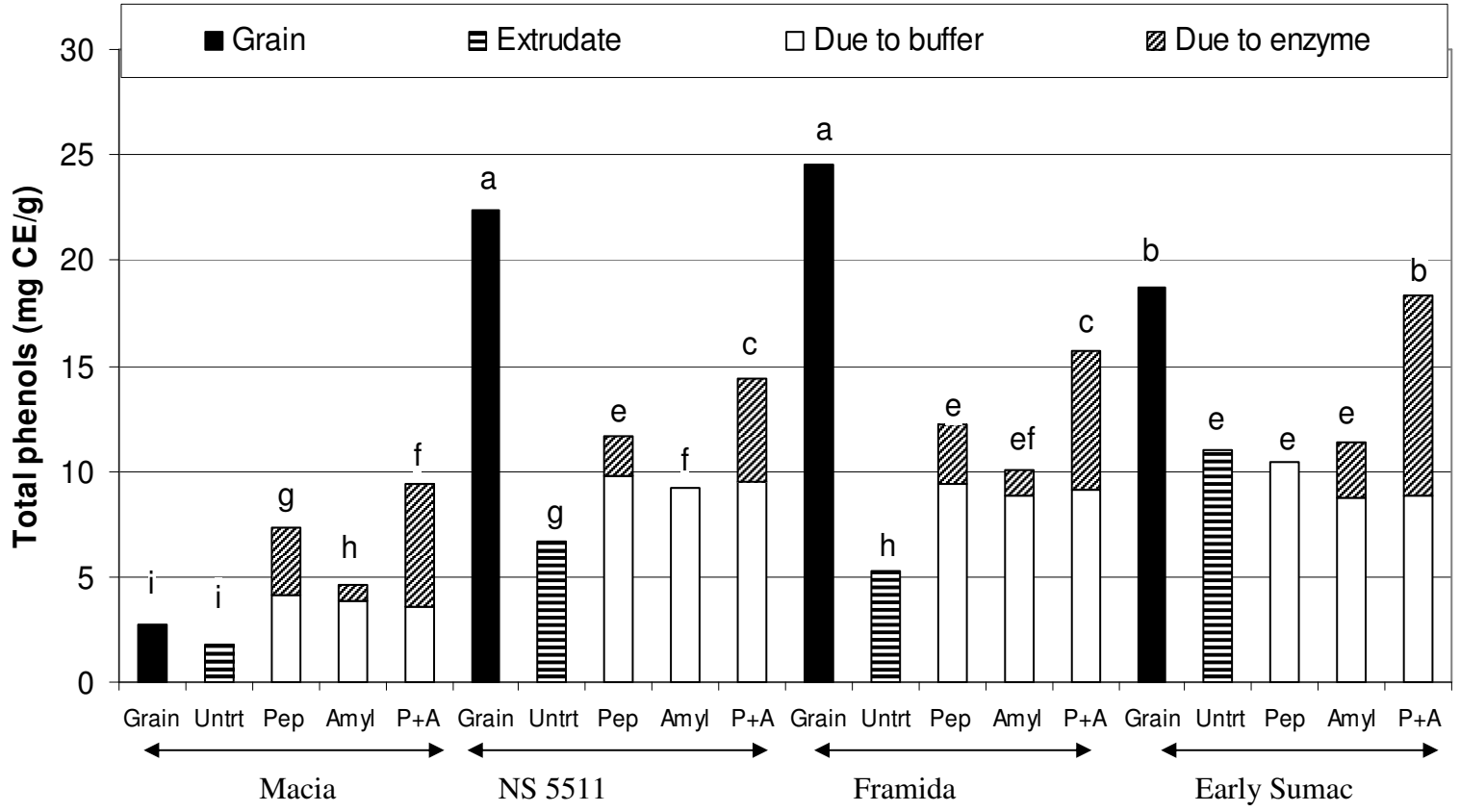

Fig. 19. Effect of enzyme treatment on total phenols of sorghum extrudates. Total phenols determined on dry basis. Bars with different letters are significantly different, P<0.05.

Abbreviations: Untrt-untreated; Amyl-amylase; pep-pepsin; P+A-pepsin, followed by amylase.

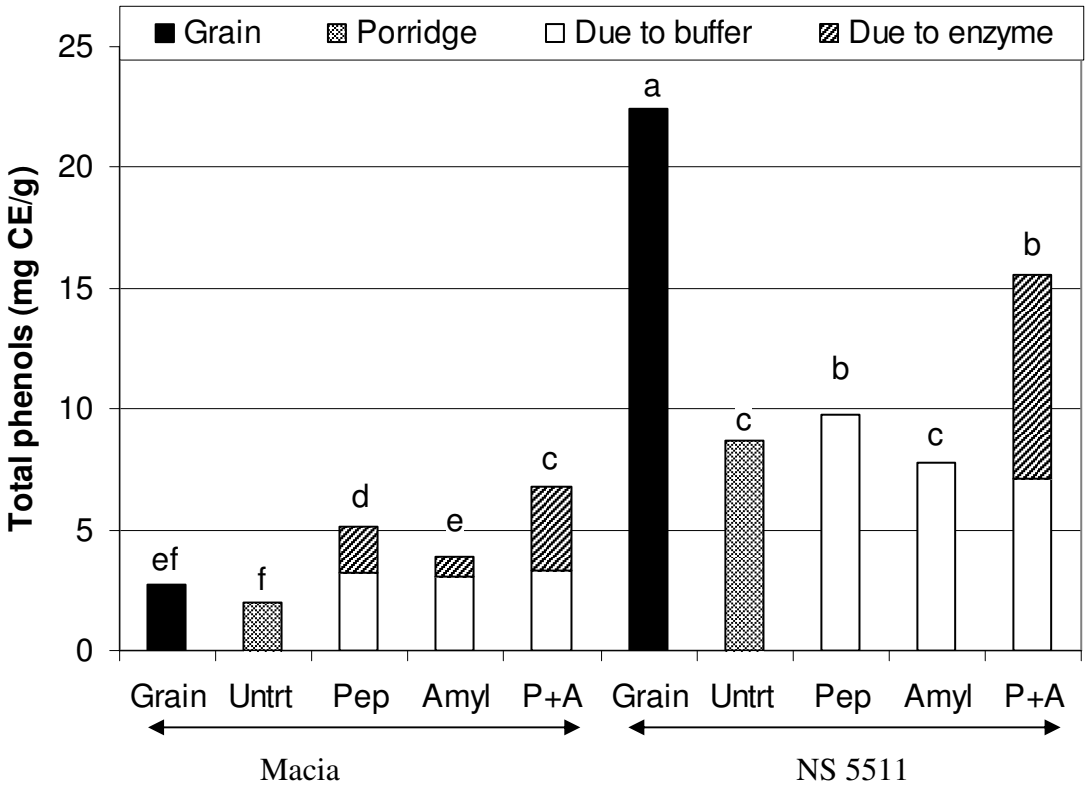

Fig. 20. Effect of enzyme treatment of sorghum porridges on total phenols. Total phenols expressed as mg CE/g, dry basis. Bars with different letters are significantly different, P<0.05.

Abbreviations: Untrt-untreated; pep-pepsin; amyl-amylase; P+A-pepsin, and then amylase.

Effect of sorghum type, processing and enzyme treatment on phenols

Pepsin followed by amylase treatments of extrudates and porridges significantly increased total phenols, compared to untreated; pepsin only and amylase only treated samples (Table XIII). As expected, the enzyme treated tannin sorghum products had higher phenols than non tannin Macia products.

Pepsin followed by amylase treated Early Sumac extrudates had significantly higher phenols than similarly treated Framida and NS 5511 extrudates (Fig. 19). Pepsin

solubilized more phenols than amylase as evidenced by significant amounts of phenols in the supernatants (Appendix Table B-1).

In porridges, pepsin followed by amylase hydrolysis of Macia and NS 5511 porridges significantly increased total phenols, compared to amylase or pepsin hydrolysis only (Fig. 20). The NS 5511 and Macia porridges and extrudates treated with pepsin, followed by amylase had higher phenol recovery than samples treated with either pepsin or amylase alone (Fig. 21). Macia extrudates had 350% phenol recovery, while the porridge had 250%. NS 5511 porridges and extrudates had average recoveries of 64 and 70 % respectively.

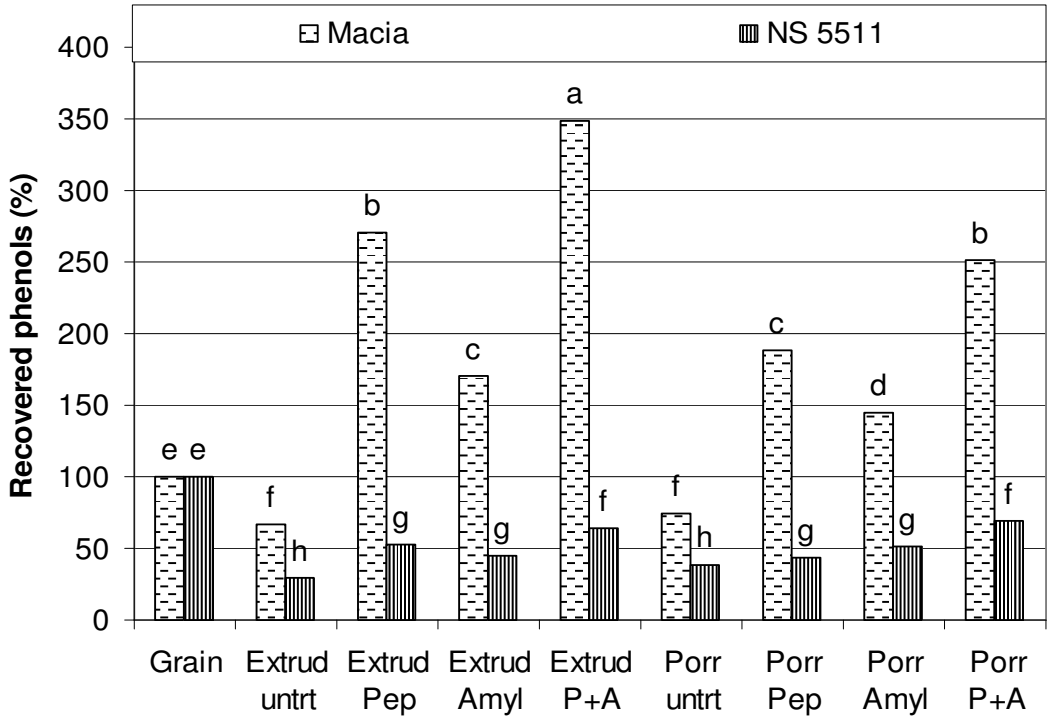

Fig. 21. Effect of sorghum type, processing and enzyme treatment on recovered phenols in sorghum extrudates and porridges. Bars with different letters are significantly different at $P<0.05$. Abbreviations: Extrud untrt-Untreated extrudates; Porr untrt-untreated porridge; Extrud/ Porr pep- pepsin treated extrudate or poridge; Extrud / Porr Amyl-amylase treated extrudate or porridge; Extrud P+A / Porr P+A- pepsin, followed by amylase treated extrudate or porridge.

Effect of sorghum type and processing on antioxidant activity of sorghum grain

Antioxidant activity was significantly affected by sorghum type, processing and enzyme treatment (Table XIV). As expected tannin sorghum grain had significantly higher antioxidant activity than the non-tannin Macia grain (Fig. 22). These sorghums had significantly different antioxidant activity ($p<0.05$). Framida grain had the highest antioxidant activity (427 μmol TE/g), followed by NS 5511 (384 μmol TE/g), then Early Sumac (262 μmol TE/g) and lastly Macia (22 μmol TE/g). Sorghum extrudates and porridges had significantly decreased antioxidant activity.

The antioxidant activities of NS 5511 extrudates and porridges were 58 and 70 μmol TE/g, representing 15 and 18% antioxidant recoveries, while Early Sumac extrudates had higher antioxidant recovery (145 μmol TE/g, 55% recovery) (Fig. 23). The Early Sumac porridges had the highest antioxidant recovery (55%). Antioxidant recovery from Macia extrudates and porridge was also very low. The antioxidants are mainly bound to the food matrix during food processing, thus reducing extractability and recovery.

The NS 5511 and Framida extrudates had lower antioxidant recovery than Early Sumac extrudates probably due to different extrusion cooking methods.

Table XIV
Effect of pepsin, amylase and pepsin followed by amylase treatment on antioxidant activity of sorghum extrudates and porridges

Treatment	Macia	NS 5511	Framida	Early Sumac	**Mean enzyme effect**
Grain	22 c	384 a	427 a	262 a	**274 A**
Extrudate	4 d	58 g	53 d	145 b	**65 C**
Porridge	1 d	70 f	118 c	145 b	**84 C**
Grain-Pepsin	231	325	-	-	
Grain-Amyl	9	125	-	-	
Grain-Pep+Amyl	118	180	-	-	
Extrudate-Pepsin	227 a	244 c	264 b	254 a	**247 A**
Extrudate-Amyl	17 c	117 e	115 c	148 b	**99** B
Extrudate Pep+Amyl	146 b	274 c	216 b	313 a	**237 A**
Porr-Pepsin	105	244	-	-	
Porr-Amyl	5	111	-	-	
Porr-Pep+Amyl	93 c	308 b	387 a	270 a	**265 A**
Mean cultivar effect	**73 B**	**236 A**	**246 A**	**246 A**	

Values within a column with different letters are significantly different, $p<0.05$.
Values for mean cultivar and enzyme effects with different upper case letters are significantly different, within the respective row and column. Mean values exclude enzyme treated grain, Macia and NS 5511 pepsin and amylase treated porridges.
[a]Antioxidant activity (ABTS method) expressed as µmol Trolox Equivalents per g (µmol TE/g), dry basis (Awika et al 2003a)
[b]- No data value.
[c] Abbreviations: Porr –porridges, pep-pepsin; Amyl- amylase

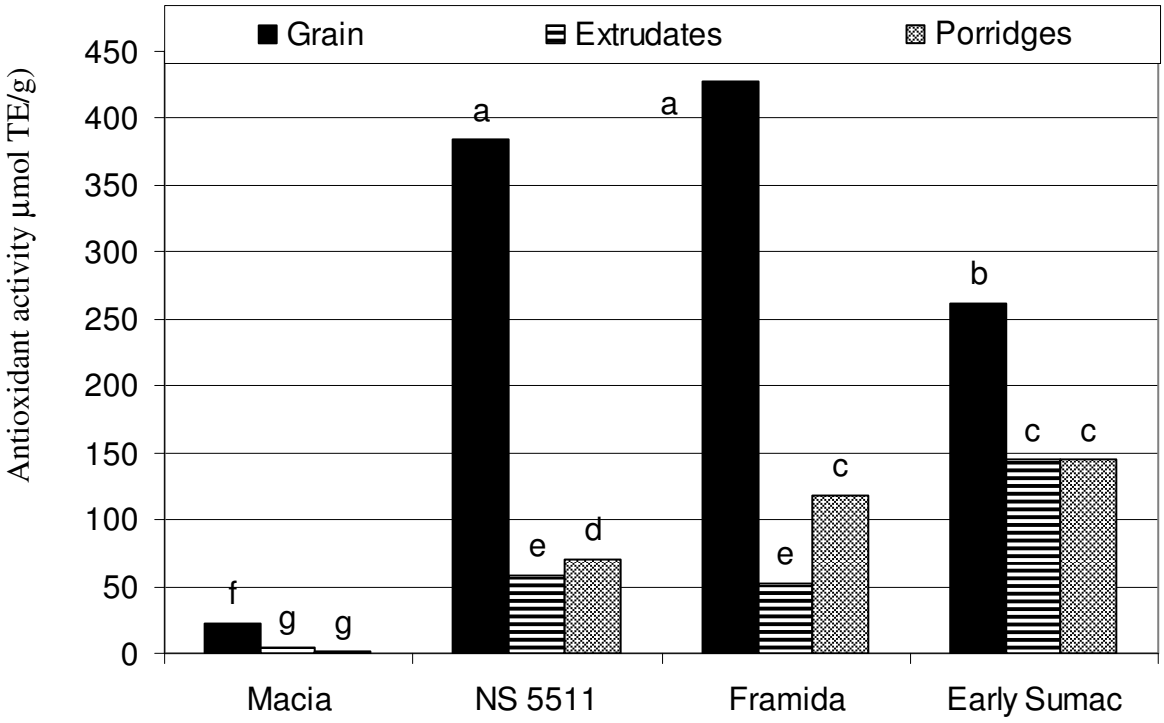

Fig. 22. Effect of sorghum type and processing on antioxidant activity of Macia, NS 5511, Framida and Early Sumac grain. Bars with the same letter are not significantly different, $p<0.05$.

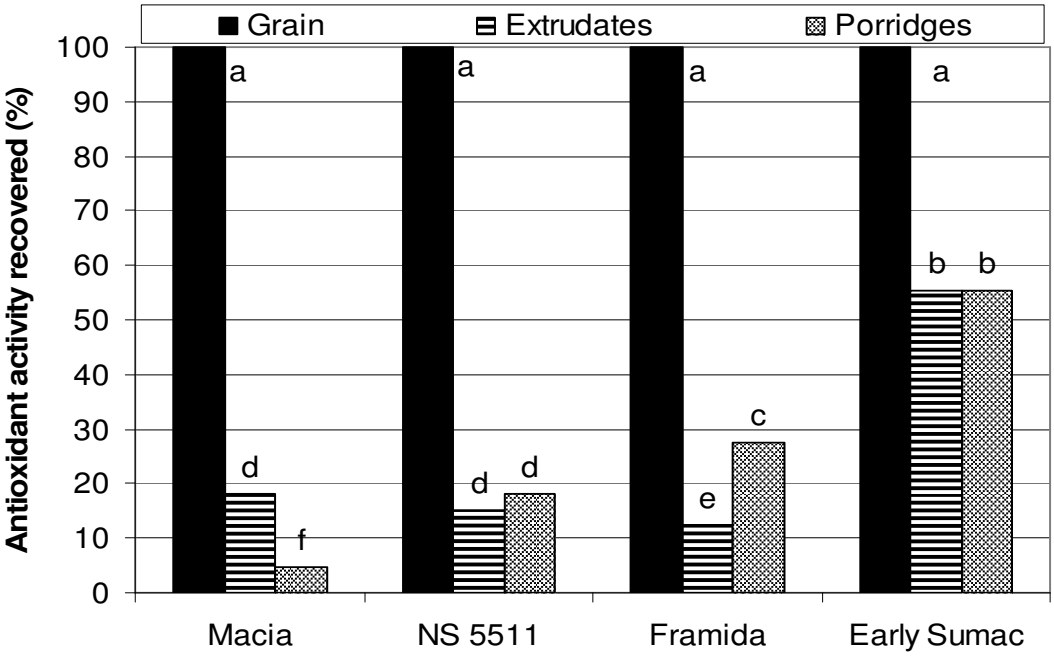

Fig. 23. Effect of processing on antioxidant recovery of sorghum extrudates and porridges. Bars with the same letter are not significantly different.

Effect of sorghum type and enzyme treatment on antioxidant activity of sorghum grain

The effect of enzyme treatment on antioxidant activity of ground, unprocessed grain was significantly affected by sorghum type (Fig. 24, Appendix Table B-2). Enzyme treatment of Macia grain significantly increased antioxidant activity, while enzyme treatment of NS 5511 decreased antioxidant activity. Pepsin or pepsin followed by amylase treatments were most effective in increasing antioxidant activity.

The antioxidant activity of ground Macia grain was increased from 22 to 231 µmol TE/g, while that of NS 5511 grain, decreased from 384 to 325 µmol TE/g. In both these treated grains, the highest level of antioxidant activity was in the supernatants, compared to residues, (223 µmol TE/g for Macia grain supernatant, compared to 8 µmol TE/g in residue) (Appendix Table B-2). Amylase treatment decreased antioxidant activity of Macia and NS 5511 grains, compared to the unprocessed grains.

The antioxidant recovered in pepsin treated Macia grain was over 1000 %, while that for combined pepsin and amylase treatment was over 500 % (Fig. 25). In enzyme treated NS 5511 grain the highest antioxidant activity was in pepsin treated samples (85% recovery).

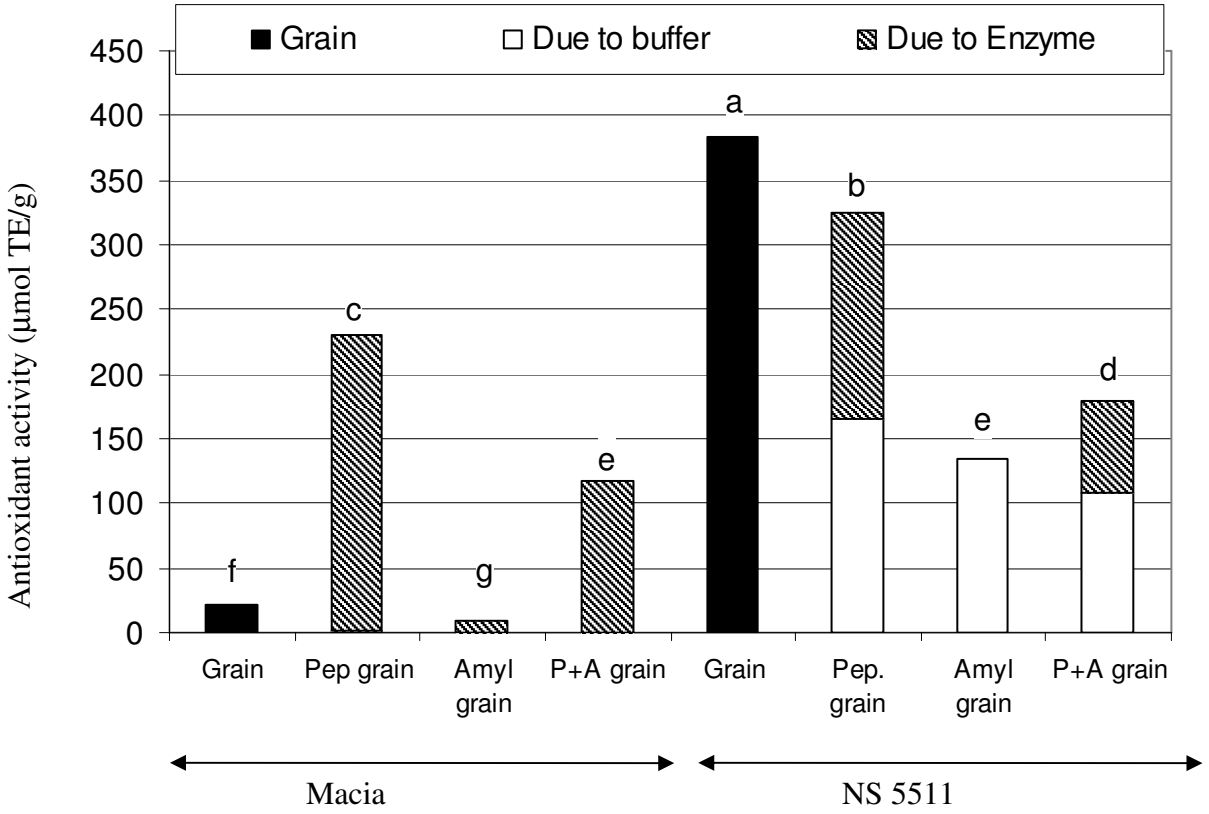

Fig. 24. Effect of enzymes on the antioxidant activity of sorghum grain. Effects due to buffer are based on results from the enzyme controls. Bars with different letters are significantly different, $p<0.05$.
Abbreviations: Amyl-amylase treated; Pep-pepsin treated; P+A- pepsin, followed by amylase treated.

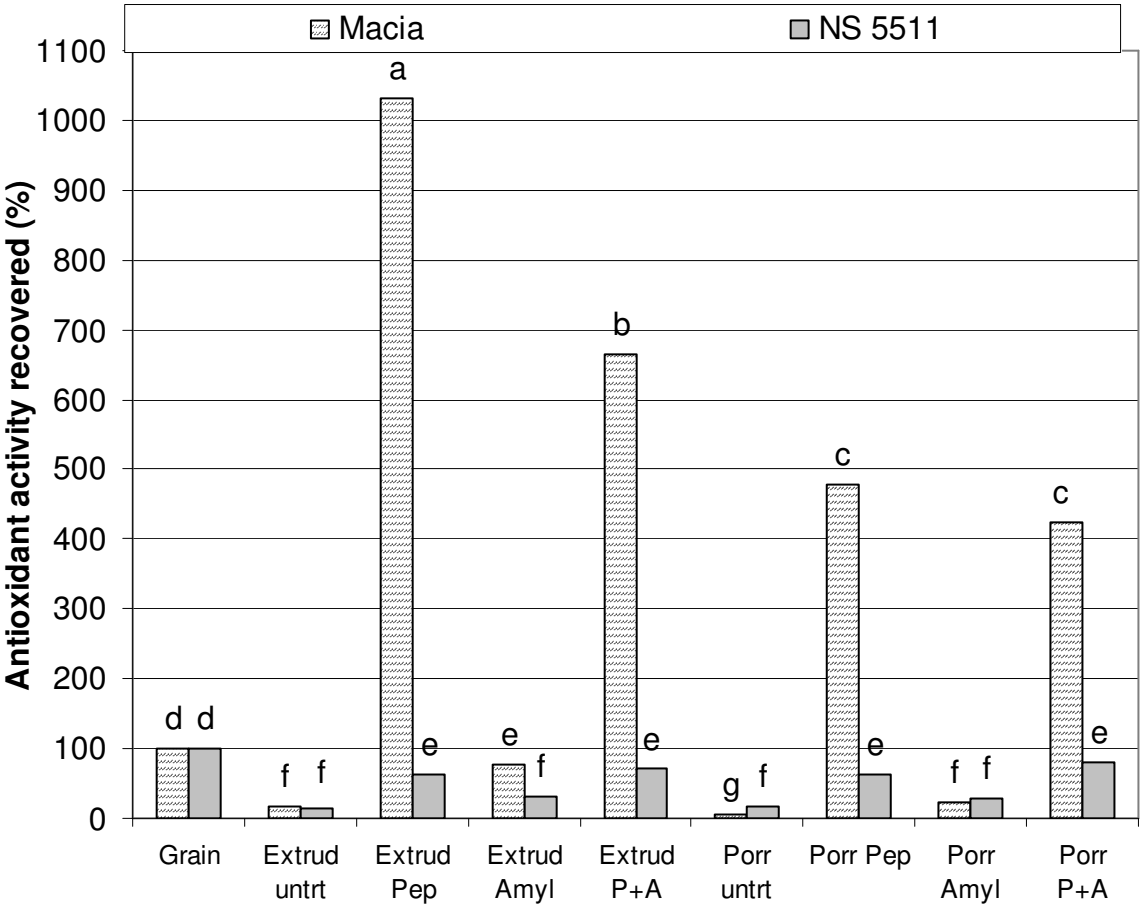

Fig. 25. Effect of enzymes on the recovery of antioxidant activity in sorghum grain (ground, unprocessed). Bars with different letters are significantly different at $P<0.05$. Abbreviations: Pep-pepsin; Amyl-amylase; P+A-Pepsin and amylase treated grains.

Effect of enzymes on the antioxidant activity of extrudates and porridges

Pepsin treatment of extrudates increased antioxidant activity compared to that of untreated extrudates (Table XIV). In all the treated extrudates, highest antioxidant activity was in the supernatants, an indication of increased solubility of antioxidants.

Pepsin treated or pepsin followed by amylase treated extrudates had significantly higher antioxidant activity than extrudates treated with amylase only (Fig. 26). Pepsin treatment increased antioxidant activity of Macia extrudates from 4 to 227 μmol TE/g, while that of NS 5511 extrudates was increased from 58 to 244 μmol TE/g. For pepsin treated tannin sorghum extrudates highest recovery was in Early Sumac extrudates (97% recovery, 254 μmol TE/g) (Table XIV). The antioxidant activities of pepsin treated Macia and tannin sorghum extrudates were not significantly different, $p<0.05$.

Pepsin and pepsin followed by amylase treated porridges showed high antioxidant activity when compared to porridges treated with amylase only (Fig. 27). The recoveries of antioxidant activity in enzyme treated Macia and NS 5511 extrudates and porridges are compared in Fig. 28. Macia extrudates had over 1000% antioxidant recovery, while NS 5511 extrudates had 64% recovery. For pepsin treated tannin sorghum extrudates highest recovery was in Early Sumac extrudates (97% recovery, 254 μmol TE/g).

The effect of pepsin, dominated over the buffer effect in the antioxidant activity of Macia extrudates, whereas in NS 5511, Framida and Early Sumac extrudates, there was notable effect of buffer. The samples incubated in pepsin buffer had increased levels of antioxidant activity due to the solubilizing effect of the low pH buffer (pH 1.5) (Appendix Table B-2).

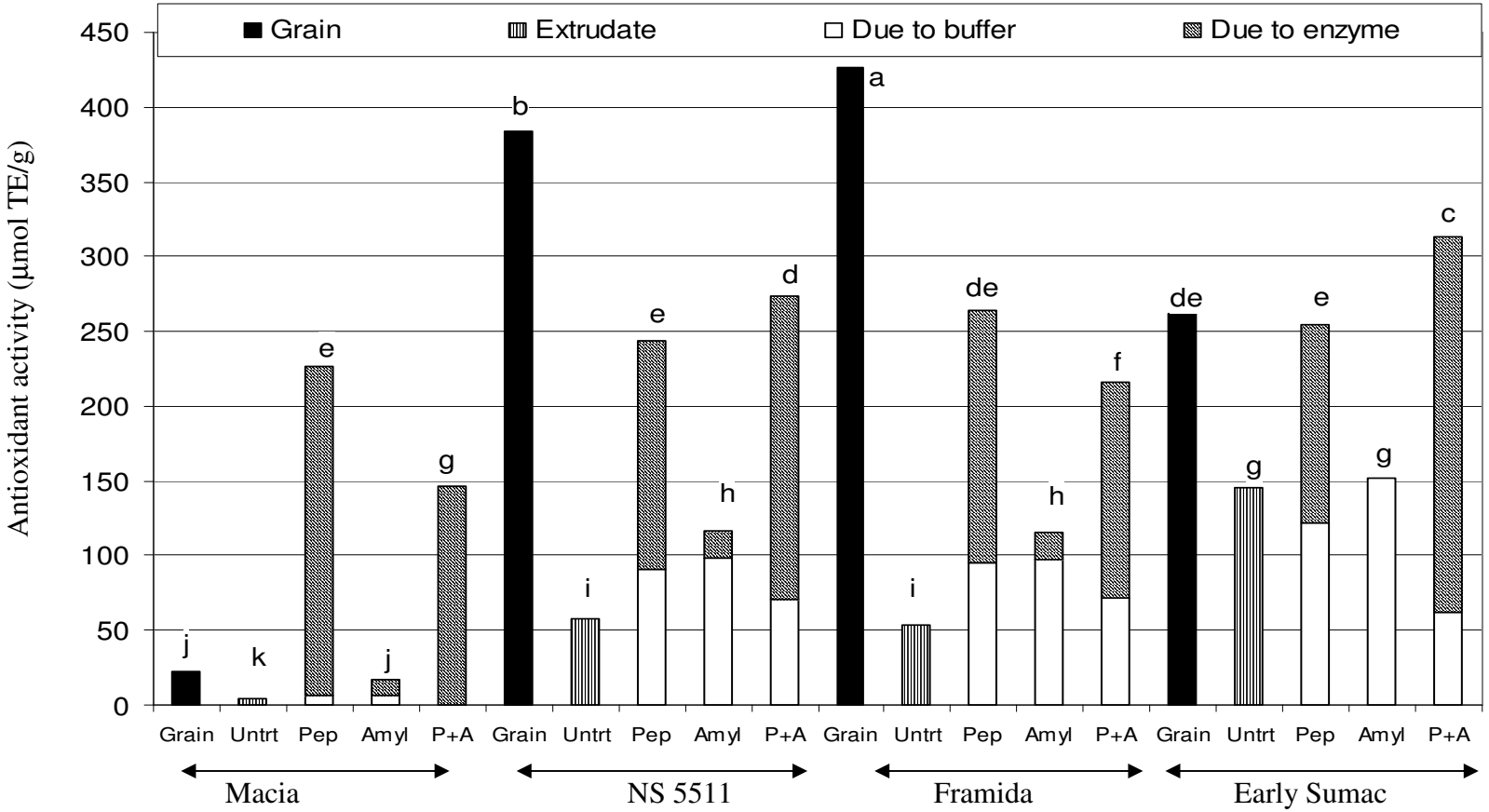

Fig. 26. Effect of enzymes on antioxidant activity of sorghum extrudates. Antioxidant activity due to buffer and enzyme effects is illustrated. Antioxidant activity expressed as µmol TE/g, dry basis.

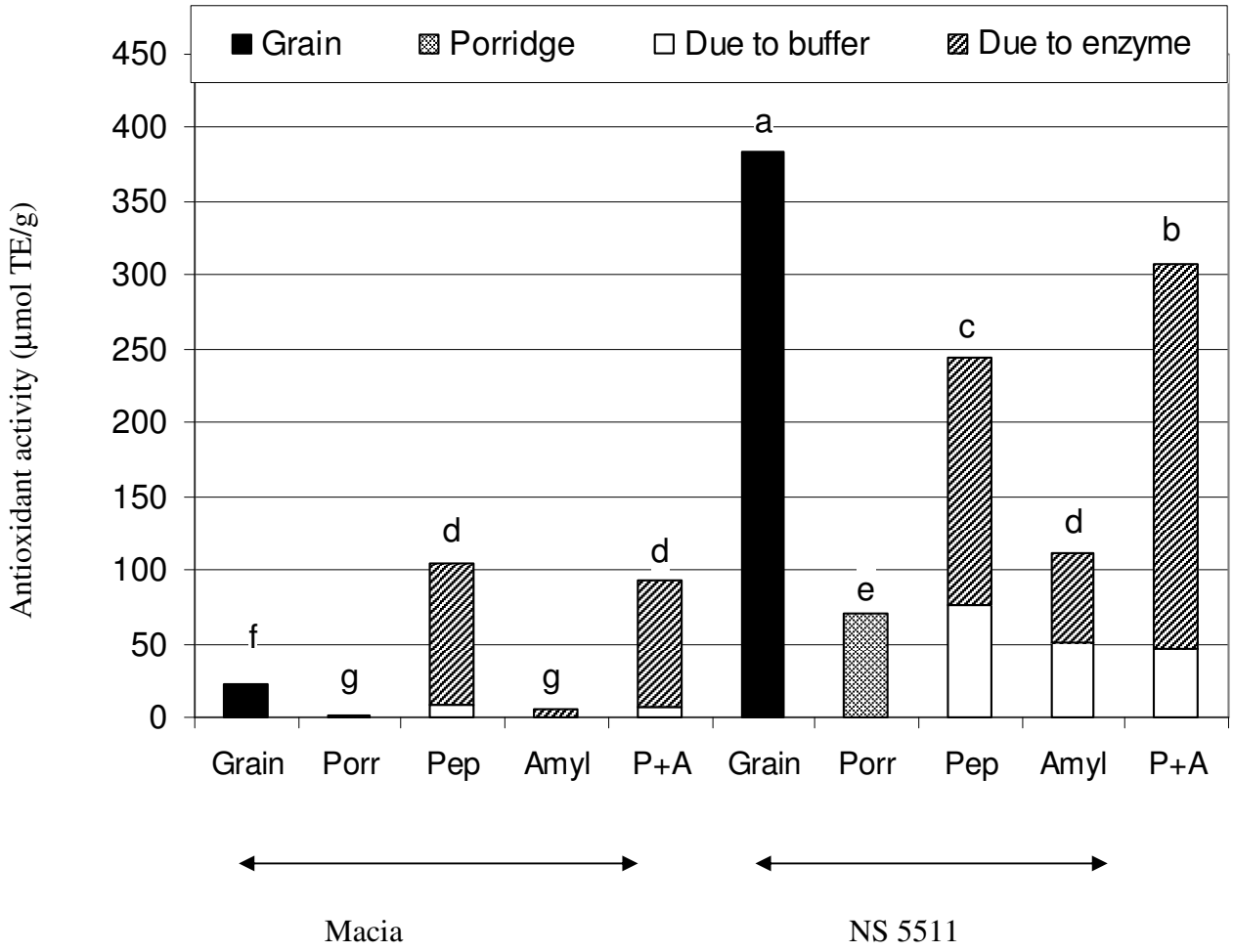

Fig. 27. Effect of enzymes on antioxidant activity of sorghum porridges. Antioxidant activity due to buffer and enzyme effects is illustrated. Bars with different letters are significantly different at $P<0.05$.

Abbreviations: Porr-porridge; pep-pepsin; amyl- amylase; P+A- pepsin and amylase

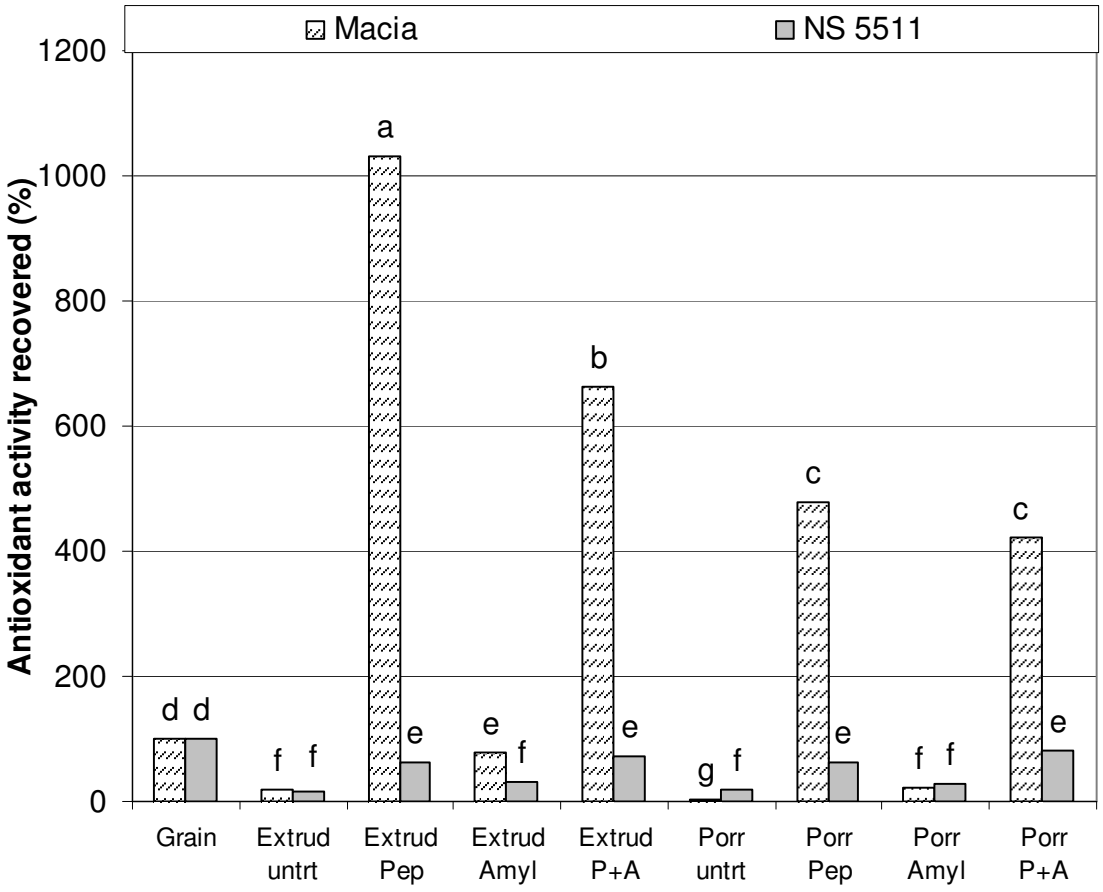

Fig. 28. Effect of sorghum type, processing and enzyme treatment on recovered antioxidants in sorghum extrudates and porridges.

Mac.-Macia ; NS-NS 5511; pep-pepsin; Amyl-amylase; P+A pepsin and amylase

Effect of pepsin and amylase treatment on tannin content of extrudates and porridges

Sorghum processing decreased tannin content in porridges and extrudates (Table XV). Macia grain had no tannins. Overall, enzyme treatment improved the extractability of phenols, both in the enzyme supernatant and in acidified methanol. For this phase of study, residues only were assessed for tannins, where reduced tannin content in the residue was an indication that tannins were solubilized in the supernatant, and thus released by enzyme activity only.

The residues from pepsin-treated extrudates had reduced tannin content compared to those from amylase treatment. This indicated that pepsin increased the solubility of tannins (Fig. 29). NS 5511 pepsin treated porridge had reduced tannin content compared to amylase treated porridge, which also indicated increased tannin solubility or reduced binding. The increase in phenols in the supernatants supports this inference.

Table XV
Effect of pepsin, amylase and pepsin followed by amylase treatment on tannin content of residues remaining after enzyme hydrolysis of extrudates and porridges

Sample	Macia	NS 5511	Framida	Early Sumac	**Mean**
Grain	ND	49.0 a	47.8 a	19.3 a	**29.0 A**
Extrudate	ND	1.9 e	0.4 d	8.6 b	**2.7** B
Porridge	ND	6.6 d	13.7 b	4.5 c	**6.2 B**
Extrudate-Pepsin	ND	0.5 e	ND	0.2 d	**0.2 C**
Extrudate-Amyl	ND	5.3 e	2.7 c	10.4 b	**4.6 B**
Extrudate Pep+Amyl	ND	3.2 e	1.1 c	10.7 b	**3.8 B**
Porr-Pep	ND	0.1 f	-	-	**0.1 C**
Porr-Amyl	ND	9.9 c	-	-	**5.0 B**
Porr-Pep+Amyl	ND	12.3 b	14.7 b	10.5 b	**9.4 B**
Mean[c]	**0 B**	**5.3** A	**4.6** A	**8.0** A	

[a]Data within the same column with different letters are significantly different, $p<0.05$. Tannins determined using the vanillin-HCl method and expressed as mg catechin equivalents (mg CE/g), dry basis.
[b]NS 5511 and Framida extrudates were produced using a twin-screw extruder, while Early Sumac extrudates were produced on a single-screw extruder.
[c]Mean cultivar effect expressed for enzyme treated samples only, data with different upper case letters are significantly different, $p<0.05$
- No data value, ND- not detected

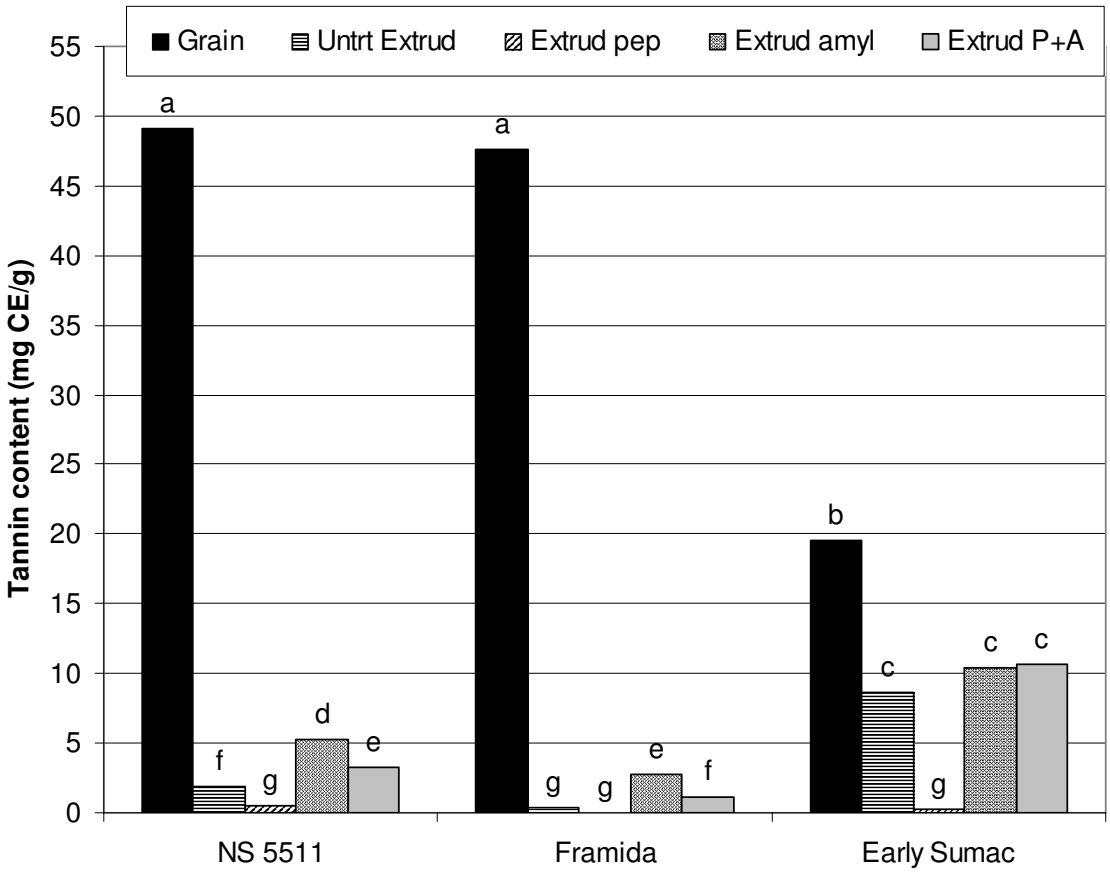

Fig. 29. Effect of enzyme treatment of NS 5511, Framida and Early Sumac extrudates on tannin content of the residues. Tannin content expressed as mg CE/g, dry basis. Bars with different letters are significantly different ($p<0.05$).

Abbreviations: Extrud-Extrudates; Pep-Pepsin treated, Amyl-amylase; P+A-pepsin followed by amylase.

Effect of enzyme treatment on bread containing white or tannin sorghum bran on phenols and antioxidant activity

The baking process decreased measurable phenols and antioxidant activity of bread prepared from flour containing tannin sorghum bran (tannin bread) or non- tannin, white sorghum bran (white bread) (Table XVI). Pepsin and pepsin followed by amylase treatments significantly increased solubility of total phenols and antioxidants in white and tannin breads. Amylase treatment reduced the solubility of tannin bread phenols, while those of white bread were not significantly affected.

Pepsin and pepsin followed by amylase treatments increased antioxidant activity, in white and tannin bread (Fig. 30). Amylase treatment significantly increased antioxidant activity of white bread, from 2 to 27 μmol TE/g, while that of tannin bread was not significantly affected. In tannin bread, higher antioxidant activity (244 μmol TE/g) was attributed to tannins derived from the added bran. Pepsin treatment of white bread increased antioxidant activity from 2 to 125 μmol TE/g, while pepsin followed by amylase treatment increased it to 146 μmol TE/g. Pepsin, and pepsin followed by amylase treated tannin bread had higher antioxidant activity (244 and 250 μmol TE/g) respectively.

The antioxidant activity recovered in pepsin and pepsin followed by amylase treated tannin bread was more that 300%, while that for similarly treated white bread was greater than 2500% (Fig 31). Higher recovery in white bread was due to its very low initial antioxidant activity in the ingredients (5 μmol TE/g), while the tannin bread ingredients had antioxidant activity of 66 μmol TE/g, derived mainly from the tannin bran.

Table XVI
Effect of enzyme treatment on solubilized phenols and antioxidant activity in bread containing 12% white or tannin sorghum bran

Treatment/ Sample	Phenols (mg CE/g)[a]		Antioxidant activity[b] (µmol TE/g)	
	Bread containing white bran	Bread containing tannin bran	Bread containing white bran	Bread containing tannin bran
Control (ingredients)[c]	2 c	6.4 b	5 c	66 b
Control (Bread)[d]	2.5 c	3.1 c	2 c	37 c
Pep and Amyl[e]	6.7 a	8.5 a	146 a	250 a
Buffer (P+A)[f]	2.0 b	2.3 d	0 c	8 d
Pepsin	4.4 b	8.7 a	125 a	244 a
Buffer (P)	1.8 c	1.7 d	6 c	15 d
Amylase	2.7 c	2.8 c	27 b	35 c
Buffer (A)	2.0 c	1.9 d	4 c	5 d

Values within the same column with different letters is significantly different, $p<0.05$.
[a]Phenols expressed as mg catechin equivalents/g (mg CE/g), dry weight basis (Folin-Ciocalteu method).
[b]Antioxidant activity expressed as µmol Trolox Equivalents per g (µmol TE/g), dry basis (Awika et al 2003a)
Solubilized or supernatant phenols and antioxidant activity is shown.
[c]Ingredients: Flour containing 12 % white or tannin sorghum bran.
[d]Untreated, freeze dried bread was incubated in buffers containing the enzymes, and residues and supernatants were separated by centrifuging.
[e]Freeze dried bread treated with pepsin, amylase or pepsin, follwed by amylase (Pep and Amyl)
[f]Buffers A, P and P+A- Enzyme control for amylase, pepsin, and pepsin followed by amylase treatments.

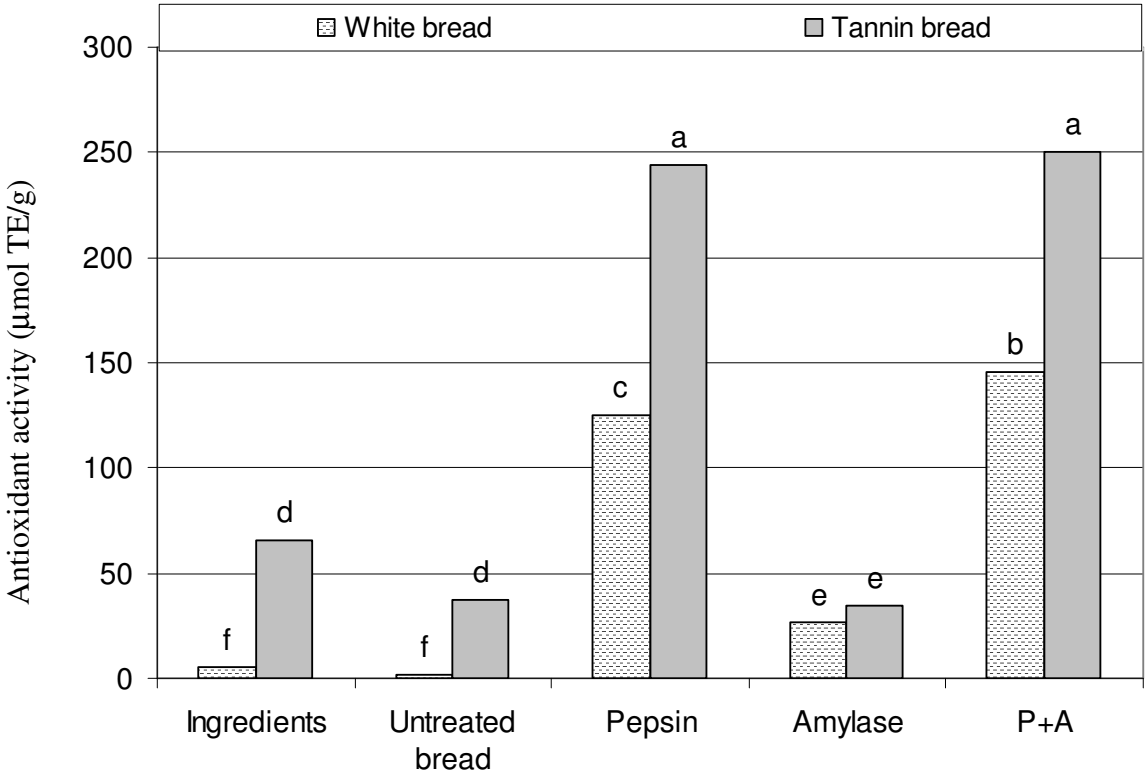

Fig. 30. Effect of enzyme treatment on antioxidant activity of bread containing white or tannin sorghum bran. Bars with different letters were significantly different, P<0.05.

Abbreviations: Ingredients –White bread ingredients were 12% white, non-tannin sorghum bran in wheat flour. Tannin bread ingredients were 12% tannin sorghum bran in wheat flour.

Pepsin treated; Amylase treated; P+A – pepsin, followed by amylase treatment

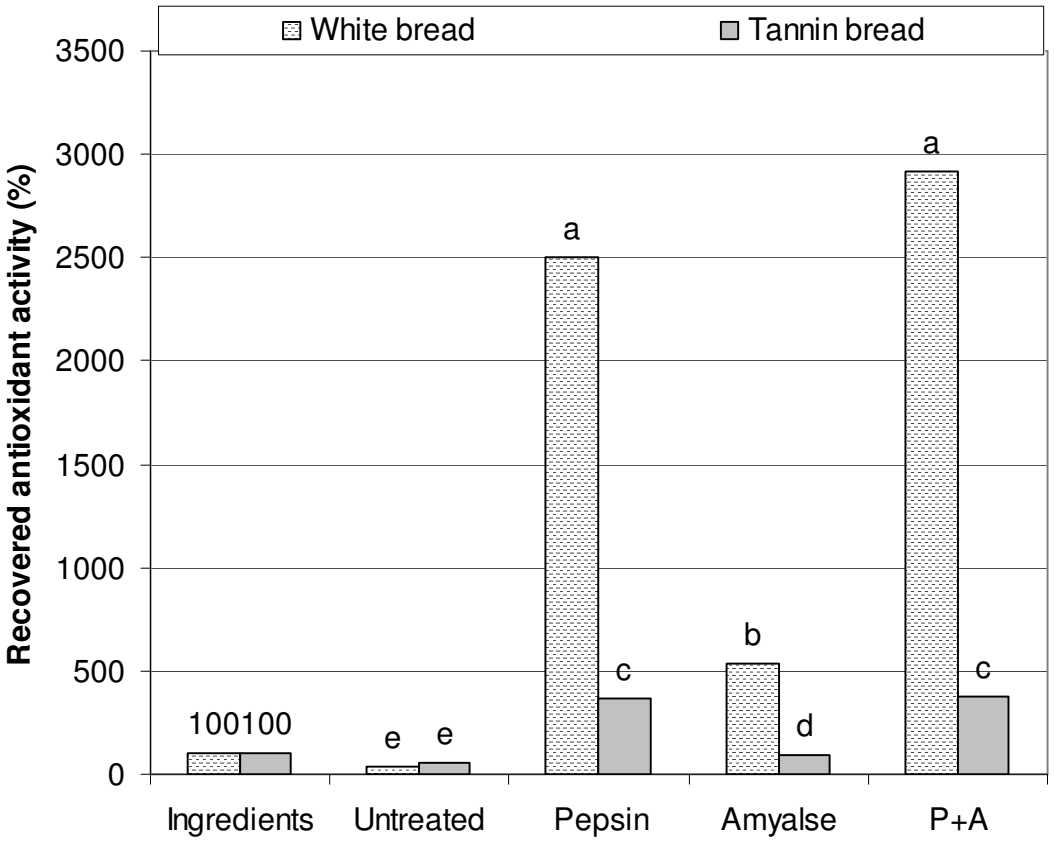

Fig. 31. Effect of enzyme treatment on recovered antioxidants in bread containing white or tannin sorghum bran. Ingredients represented 100% recovery. Bars with different letters are significantly different at $P<0.05$.

Abbreviations: Ingredients –White bread ingredients were 12% white, non-tannin sorghum bran in wheat flour. Tannin bread ingredients, were 12% tannin sorghum bran in wheat flour.

Pepsin treated; Amylase treated; P+A – pepsin, followed by amylase treatment

DISCUSSION

The porridges, extrudates and bread presented different processing systems and possible phenol interactions. The extrudates were produced at low moisture content (14-18%), high temperatures (150-160^{o}C), under high pressure and shear. The porridges were prepared under high moisture (80-85%), lower temperatures (at the most 100^{o}C), and low shear. Thus more phenol interactions were expected in extrudates due to intimate mixing in the extruder barrel, and stabilization of hydrophobic associations at high temperatures (Wieser 2007). In porridges phenol interactions were favored by the high moisture, while in bread, the long processing time and the hydrophobic wheat proteins may have increased protein-phenol interactions with an overall reduction of phenols and tannins.

The observed dry matter loss was mostly a function of enzyme treatment and processing method. The extrudates had significantly higher dry mass loss than the porridges, where hydrolysis may have been limited by retrogradation which resulted in slowly digestible resistant starch (RS) in porridge. The extent of retrogradation was not measured in this study. Extrusion cooking of cereals has been observed to increase the proportion of rapidly digestible starch, while that of slowly digestible starch is decreased (Sun et al 2006). Extrusion cooking disrupts associations between protein and starch, reduces lipid-starch interactions, denatures protein, ruptures starch granules and gelatinizes starch, thus increasing enzyme-susceptibility of both starch and protein (Alonso et al 2000). Samples treated with amylase or a combination of pepsin followed by amylase had the highest dry matter loss while pepsin treated samples had lower dry matter loss, but increased phenol solubility.

The increase in phenol content and antioxidant activity of pepsin treated sorghum products indicated that the phenols, especially the tannins were released when protein was hydrolyzed, or there was release of peptides and aromatic amino acids such as tyrosine. Although pepsin has broad specificity, studies show that it preferentially cleaves peptide bonds with aromatic amino acids (Schnaith 1989), thus effectively disrupting hydrophobic regions of the protein. The peptides and aromatic amino acids

that are released by pepsin hydrolysis react with Folin Ciocalteu (Schnaith 1989) and ABTS reagents, and thus may increase phenol content and antioxidant activity. Other substances that interfere with the Folin reagent include sugars, aromatic amines, sulfur dioxide, ascorbic acid, organic acids and Fe (II) (Roura et al 2006).

Since pepsin hydrolysis potentially decreases hydrophobic regions of protein, it can be inferred that hydrophobic associations of proteins and tannins are also weakened, and tannins may be released into solutions. Some of the strongest links between protein and polyphenols are hydrophobic links (Renardi et al 2001), in addition to weaker hydrogen bonds (Porter 1992). The challenge is to determine the contribution of tannins, peptides and amino acids to overall increase in phenols and antioxidant activity. Roura et al (2006) reported that polyphenols from ingested polyphenol-rich food such as cocoa beverage could be accurately determined if biological samples such as urine, were cleaned up using solid phase extraction. Roura et al (2006) applied acidified patient urine to activated Waters Oasis HLB cartridges, and washed the samples with 1.5M formic acid followed by a 95:5 mixture of water and methanol. Polyphenols were eluted with a 1:1 mixture of methanol and formic acid. The supernatant from enzyme treated samples could be cleaned up in a similar way. The phenols could be identified using liquid chromatography-tandem mass spectrophotometry.

The release or solubilization of phenols and antioxidant activity by amylase treatment was not as significant as that of pepsin treatment, probably demonstrating the minor role of starch–phenol interactions in reduced antioxidant activity. Phenol solubility could be reduced by being trapped in the starch matrix, like in white bread where amylase treatment increased phenol solubility. In addition, phenols may form complexes with cell wall material (Renard et al 2001).

Enzyme treatment of unprocessed sorghum grain presented a slightly different picture, and there were indications of enzyme inhibition, observed with enzyme treated NS 5511 grain and porridge. Tannins bind to protein forming undigestible complexes (Scalbert et al 2000, Papadopoulou and Frazier 2004). Pepsin, and pepsin followed by amylase treated Macia grain had significantly increased phenol and antioxidant activity,

well above that present in the unprocessed grain, while enzyme treated NS 5511 grain had reduced phenol and antioxidant activity compared to unprocessed grain. The unprocessed grains were generally less susceptible to enzyme hydrolysis and had low dry matter loss compared to processed products. Extrusion cooking and porridge-making increase starch solubility through gelatinization, while heat will denature protein, causing unfolding of the peptide chains, making the peptide bond more accessible to pepsin.

There was no significant enzyme inhibition in processed tannin sorghum products, for example the pepsin treated tannin and non-tannin extrudates had similar dry matter loss (Table XII). Some studies have suggested that enzyme activity can be preserved when tannins bind to proteins that have higher affinity than the enzyme itself (McGrath et al 1993). Prolamins of sorghum have high affinity for tannins (Emmambux and Taylor 2003), and could have protected the enzymes from inhibition. In addition, at higher sorghum dilutions, 20-28% solids concentration, inhibition of enzyme activity was minimal (de Jong et al 1987).

Pepsin or pepsin, followed by amylase treatment of bread also significantly increased antioxidant activity, and as mentioned before, there is the possible role of tyrosine and other amino acids with antioxidant activity, as well as the release of bound phenols. Hydrolysis of gluten is known to increase antioxidant activity. Wang et al (2007) reported high antioxidant activity in wheat gluten hydrolyzed using papain. Other amino acids with high antioxidant activity include histidine, methionine and cysteine (Wang et al 2007).

Although antioxidant activity of pepsin treated tannin extrudates was not significantly different from that of non tannin Macia extrudates, there is still a possibility that in tannin products there was considerable masking effect due to tannin interactions with protein, peptides or amino acids. Arts et al (2001) reported reduced antioxidant activity due to masking, while Riedel and Hagerman (2001) reported antioxidant activity (ABTS scavenging ability) of soluble tannin protein complexes. The ABTS scavenging ability was dependent on protein isoelectric point, and tannin-protein complexes were

expected to behave in a similar way. Proteins are least soluble close to their isoelectric points.

The role of pepsin, although it was significant in sorghum products, compared to tannin sorghum grain, there was also solubilization of antioxidants and phenols by the low pH pepsin buffer (KCl-HCl, pH 1.5). In simulated gastric juice, Spencer et al (2000) observed possible depolymerization of tannins by low pH, thus an increase in solubility. The amylase treatment hydrolyzed starch and may have released some phenols entrapped in the food matrix, as was observed for white bread. The higher pH of the amylase buffer, Tris maleate buffer, pH 6.9, had minimum effect on phenols and antioxidant solubility. Generally, at higher pH condensed tannins polymerize and become less soluble (Porter 1992).

Evidence from this research suggests that pepsin or other proteases could enhance antioxidant activity of processed samples. Protein hydrolysates have been used to retard lipid oxidation (Wang et al 2007), and there is potential benefits from antioxidant amino acids like tyrosine. Kitts and Weiler (2003) have reviewed bioactive proteins and peptides; some of the beneficial properties mentioned include antioxidant activity, antihypertensive activity and immunostimulating activity to name a few. Concerning cereals, they mention that hydrolysis of α-zein by thermolysin (a heat resistant bacterial protease) yields three types of antihypertensive peptides, referred to as angiotensin I-converting enzyme (ACE) inhibitors. The common ACE-inhibitory peptides are usually rich in hydrophobic amino acids, short chain, two to nine amino acids, and resistant to further digestion (Kitts and Weiler 2003). The sorghum kafirins are similar to α-zein in corn because they are rich in proline residues.

In terms of antioxidant activity, peptides appear to have similar advantages to those of phenols, mainly retarding peroxidation of lipids or fatty acids (Wang et al 2007), and could have synergestic effects with other antioxidants such as tocopherol, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Kitts and Weiler 2003). The beneficial health benefits of flavonoids including procyanidins are also attributed to antioxidant activity. In addition, there is also evidence that flavonoids

activate antioxidant enzymes such as glutathione –s-transferase, catalase (Chung et al 1998); and will inhibit oxidases such as lipoxygenases and cycloxygenases (Heim et al 2002, Ferruzi and Green 2006). In some *in vitro* studies, procyanidins were observed to inhibit growth of colon, breast and prostate cancer cells (Bawadi et al 2005), cell death was also observed to occur. According to Visioli et al (2000), rather than a single dose of antioxidant nutrients, healthful food would provide a mixture of bioactive micronutrients, which together would provide the observed protection from coronary heart disease or cancer, for example.

Enzyme treatment of processed foods could be a relevant step in demonstrating potential bioavailability of antioxidants *in vivo*. Although the study was done *in vitro*, there are strong indications that reduced pH and pepsin in the stomach solubilizes antioxidants in processed cereal foods. Digestion has potential to improve antioxidant bioavailability of processed foods, either through the release of antioxidant amino acids or freeing up of bound phenols. Microbial activity in the colon could also play a role in breakdown of tannin-protein polymers (Deprez et al 2000).

Conclusion

The interpretation of antioxidant activity in pepsin-hydrolyzed tannin sorghum is complicated by the production of peptides and amino acids that have antioxidant activity. Further work would thus attempt to fractionate supernatants using techniques such as solid phase extractions, and then quantify antioxidant contribution from either peptides or phenols. Phenols would further be identified using a combination of HPLC and mass spectrometry.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The *in vitro* findings of the first two phases of this research inferred that antioxidant activities of different sorghum types were modified by processing. The tannin sorghums had higher phenol content and antioxidant activity than non-tannin sorghums. Processing by extrusion cooking and porridge-making significantly reduced measurable phenols, tannins and antioxidant activity. Antioxidant activity of sorghum was highly correlated with phenol and tannin content ($r = 0.90$, $r = 0.95$ respectively).

The modification of antioxidant activity of sorghum products can be explained by phenol interactions with the food matrix, particularly with protein. Reports exist on effect of proteins on antioxidant activity of polyphenols. Arts et al (2001) reported reduced antioxidant activity when phenols were bound to protein; while Riedel and Hagerman (2001) reported that even when bound to protein, tannins retained some of their antioxidant activity. The greatest reduction in antioxidant activity was observed in tannin sorghum products; we hypothesized that condensed tannins because of their large molecular size, formed insoluble complexes by interaction with protein. They interact with protein via hydrogen bonding; the strongest interactions are the hydrophobic interactions with proline rich protein residues, like sorghum kafirins (Emmambux and Taylor 2003). The greatest decrease in procyanidins was in bread containing tannin sorghum bran. Bread was a different system because of the highly hydrophobic gluten network, which probably interacted with procyanidins from the bran. Other interactions with food matrix cannot be ruled out, for example carbohydrate-procyanidin interactions have been reported. So far the most studied interactions are limited to some polyphenols and proteins, thus more research is needed in this area.

The effect of processing on procyanidins was determined using normal-phase HPLC. Generally, extrudates and porridges had lower amounts of procyanidins compared to the grain, and the most decrease was in procynaidin polymers, DP>8. This was an indication that these polymers interacted with the food matrix, and were

insolubilized. The procyanidin (PC) oligomers and monomers were not significantly affected by processing, with extrusion increasing the PC monomer content. These findings confirm those of Awika et al (2003b), where the amount and proportion of low molecular weight PCs increased on extrusion cooking of tannin sorghum. The high friction and shear generated in the extruder barrel probably causes molecular fragmentation. The increased quantity of low molecular weight PC may potentially increase bioavailability of PCs. The bioavailability of PCs is limited by large molecular size; Deprez et al (2000) observed that PC monomers up to trimers were absorbed *in vitro* by intestinal cell monolayer.

Since there was overwhelming evidence that in processed food procyanidins were less extractable, the next step was to determine what would happen to the procyanidins once food is digested in the gastrointestinal tract. In this study, we hypothesized that since procyanidin associations with the food matrix are mostly non-covalent, it is possible that these associations could be disrupted during digestion and recovery of antioxidant activity and phenols improved. The Goni et al (1997) method for estimating glycemic index of food was used for mimicking the digestion process. The extrudates, porridges and bread were treated with pepsin, followed by amylase. Samples were treated with pepsin alone, while others were treated with amylase alone. Generally samples treated with pepsin and combination of pepsin and amylase had increased phenol content and antioxidant activity, compared to the untreated products. Amylase treatment alone did not significantly increase recovery of phenols and antioxidants compared to samples hydrolyzed using pepsin alone.

Pepsin hydrolysis, either alone or in combination with amylase introduced another dimension to the analysis, that of amino acid and peptides from protein hydrolysis. Aromatic amino acids like tyrosine and tryptophan, or peptides carrying the amino acids are detected by the Folin Ciocalteu method (Schanith 1989), and in addition also exhibit antioxidant activity (Wang et al 2007). Amino acids and peptides may have contributed to significantly increased phenols and antioxidant activity of enzyme treated samples where phenol content was originally low, like the non-tannin Macia and bread

containing white sorghum bran. The release of bound phenolics, due to weakening of hydrogen or hydrophobic associations is another possibility. Protein hydrolysates were found to be effective antioxidants against peroxidation of lipids and fatty acids (Wang et al 2007). The antioxidant recovery in hydrolyzed processed foods is an indicator of potential benefits from the digestion of food, when antioxidants are released to actively scavenge dietary free radicals either introduced to or created in the alimentary system.

Further research should be done to determine the contribution of protein hydrolysates and that of phenolic compounds to overall antioxidant activity. Several epidemiological and *in vitro* studies have demonstrated potential health benefits of phenolic compounds like flavonoids and procyanidins that are not just related to their antioxidant properties. For example numerous flavonoids have been shown to alleviate oxidative stress by inducing glutathione S-transferase (GST), an enzyme proposed to protect cells against free radical damage (Ross and Kasum 2002). Procyanidin oligomers isolated from black beans have been shown to inhibit the proliferation of colon, breast and prostatic cancer cell lines (Bawadi et al 2005).

Suggested future work would therefore be to clean up supernatants from enzyme hydrolysis, for example using a solid phase extraction method as described by Roura et al (2006), determine phenols and confirm identify of phenols using HPLC methods combined with spectroscopy.

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APPENDIX A

EFFECT OF SORGHUM TYPE AND DECORTICATION ON EXTRUSION COOKING AND PORRIDGE COLOR

Water absorption index (WAI)

Finely milled grain and extrudate samples (2.5 g) were placed in centrifuge tubes and then suspended in 30 ml distilled water at 25^{o}C, and shaken for 30 minutes at low speed in an Eberbach shaker (Eberbach Corp., Ann Arbor, MI). The tubes were centrifuged at 3000 rpm for 10 minutes using the Beckman Model TJ-6 centrifuge (Beckman Instruments Inc., Spinco Division, Palo Alto, CA). The tubes were weighed before and after decanting the clear supernatant (this was retained for WSI). WAI was calculated as gram of adsorbed water per g dry sample.

Water solubility index (WSI)

The supernatant from WAI measurement was evaporated in an air oven at 150^{o}C overnight, and WSI was calculated as dry residue divided by the total dry matter in the original 2.5 g sample.

Expansion ratio (ER)

The expansion ratio was determined by measuring the diameter of the extrudate (mean of ten measurements) and the diameter of the die. It was expressed as a ratio of the measured diameters:

$$ER = \frac{\text{Diameter of extrudate}}{\text{Diameter of die}}$$

Table A-1
Effect of decortication on expansion ratio (ER), water absorption (WAI) and solubility indexes (WSI) of extruded sorghum

Variety	Treatment	Water absorption index (g/g)	Water solubility index (g/100g)	Expansion Ratio[a]
Macia	Whole grain	2.54 ± 0.1	3.44	
	Decorticated grain	2.03 ± 0.04	4.44 ± 0.01	
	Whole extruded	4.29 ± 0.3	32.95 ± 0.4	5.80 ± 0.3
	Decorticated extruded	6.45 ± 0.1	10.16 ± 0.3	6.50 ± 0.2
NK283	Whole grain	2.10 ± 0.2	3.76 ± 1.2	
	Decorticated grain	2.12 ± 0.1	2.67 ± 0.06	
	Whole extruded			6.32 ± 0.4
	Decorticated extruded	6.02 ± 0.2	11.52 ± 0.7	5.7 ± 0.2
Red Swazi	Whole grain	2.97 ± 0.1	3.83 ± 0.03	
	Decorticated grain	2.31 ± 0.1	2.93 ± 0.4	
	Whole extruded	4.34 ± 0.2	34.14 ± 0.02	6.08 ± 0.3
	Decorticated extruded	5.84 ± 0.4	21.24 ± 1.7	6.41 ± 0.6
NS5511	Whole grain	2.62 ± 0.05	3.45 ± 0.01	
	Decorticated grain	2.37 ± 0.3	3.19 ± 0.1	
	Whole extruded	4.43 ± 0.08	34.20 ± 0.07	5.95 ± 0.3
	Decorticated extruded	5.41 ± 0.03	28.38 ± 1.3	6.50 ± 0.3
Framida	Whole grain	2.71 ± 0.03	3.94 ± 0.01	
	Decorticated grain	2.36 ± 0.09	2.54 ± 0.1	
	Whole extruded	4.65 ± 0.04	30.98 ± 0.2	5.50 ± 0.2
	Decorticated extruded	5.62 ± 0.4	21.03 ± 2.4	6.05 ± 0.4

[a]Expansion ratio is expressed for extruded grain only

Table A-2
L*a*b* values of milled whole and decorticated grain and porridges

	Whole			Decorticated		
Sample	L*	a*	b*	L*	A*	b*
Macia kernel	67.0 a	3.1 a	18.9 ag			
Macia meal/flour	78.7 b	0.9 b	14.5 b	83.2 a	0.2 a	12.6 a
Macia porridge	61.0 c	2.2 c	21.9 c	67.0 b	0.1 a	17.4 b
NK 283 kernel	49.8 d	12.5 di	18.1 di			
NK 283 meal/flour	64.0 e	4.6 e	11.4 ef	84.1 c	0.2 b	11.9 c
NK 283 porridge	38.7 f	10.3 f	11.0 ef	58.4 d	3.9 c	17.3 b
Red Swazi kernel	48.8 g	10.7 g	18.6 agi			
Red Swazi meal/flour	66.9 a	6.0 h	11.3 ef	69.9 e	3.2 d	10.2 d
Red Swazi porridge	31.8 h	10.4 f	6.6 h	48.3 f	9.8 e	11.3 e
NS 5511 kernel	45.4 i	12.4 di	18.4 dgi			
NS 5511 meal/flour	58.8 j	5.6 j	10.8 f	77.7 g	2.7 f	10.6 f
NS 5511 porridge	34.2 k	12.8 k	9.4 j	48.4 f	7.5 g	13.7 g
Framida kernel	45.2 i	12.7 dk	17.2 k			
Framida meal/flour	70.1 l	5.2 m	10.0 l	79.8 h	3.0 d	9.0 h
Framida porridge	32.4 m	11.1 n	7.7 m	42.7 i	9.9 h	11.3 e

Data values within a column with the same letter are not significantly different at $p<0.05$

Effect of decortication on extrusion cooking

The extrusion cooked decorticated sorghum fractions had higher expansion ratio (ER) (), water absorption indexes (WAI) (Table A-1**),** and lower water solubility indexes (WSI). The wholegrain extrusion cooked sorghum had lower WAI, and higher WSI, and comparatively lower ER than the decorticated extrudates.

A High ER is desirable in the production of ready to eat foods, since it is an indication of higher degree of starch gelatinization, thus it can be deduced that decorticated grain had higher degree of cook than whole grain. A high WSI is due to water soluble components in whole grain extrudates. The high shear conditions of extrusion cooking could also lead to the degradation of biopolymers such as starch, into smaller molecules that are soluble. The general trend is that as WSI decreases, WAI increases (Fellows 2000), this trend was observed in this study, where decorticated grain extrudates had lower WSI and higher WAI.

Sorghum grain and product color

The cooked products were darker than the flour, lower L* values (Table A-2). The porridges, with exception of decorticated Macia, were more red than the flours (higher positive a* values). All the sorghums had more yellow than blue (positive b* values) flours and porridges. The color changes from the meal to the porridges can be explained by possible increased pigment extraction during cooking, as well as chemical interactions of the various pigments and also Maillard reactions.

APPENDIX B

EFFECT OF PEPSIN AND AMYLASE HYDROLYSIS ON ANTIOXIDANT ACTIVITY OF SORGHUM GRAIN AND PRODUCTS

Table B-1
Effect of pepsin and amylase treatments on total phenols in sorghum grain, extrudates and porridges

		Macia			NS 5511			Framida			Early Sumac		
Treatment	Sample	Grain	Extrud	Porr	Grain	Extrud	Porr	Grain	Extrud	Porr	Grain	Extrud	Porr
Control	**Untrd**	**2.7 d**	**1.8 e**	**2.0 d**	**22.4 a**	**6.7 d**	**8.7 d**	**24.5 a**	**5.3 d**	**14.4 b**	**18.7 a**	**11.0 b**	**11.2 b**
Pepsin[b]	Res	2.3 d	1.6 e	1.6 d	7.4 g	4.3 e	4.1 g		3.8 e			4.7 f	
Pepsin	Sup	5.3 c	5.7 c	3.5 c	10.0 e	7.5 d	5.6 f		8.4 c			5.4 f	
Pepsin	**Total**	**7.7 a**	**7.3 b**	**5.1 b**	**17.4 b**	**11.7 b**	**9.8 c**		**12.2 b**			**10.2 c**	
Buffer (P) [c]	Res	1.9 d	1.8 e	1.6 d	10.2 e	4.8 e	4.9 fg		4.2 e			7.4 e	
Buffer (P)	Sup	2.2 d	2.3 e	1.7 d	7.1 g	5.0 e	3.5 h		5.1 d			3.0 g	
Buffer (P)	**Total**	**4.1 b**	**4.1 d**	**3.2 c**	**17.4 b**	**9.8 c**	**8.4 d**		**9.4 b**			**10.4 c**	
Amylase	Res	1.8 d	2.2 e	2.2 d	8.4 f	5.5 e	8.2 d		5.1 d			3.0 g	
Amylase	Sup	2.3 d	2.4 e	1.7 d	5.0 h	4.5 e	3.2 h		5.1 d			8.7 d	
Amylase	**Total**	**4.0 b**	**4.6 d**	**3.9 c**	**13.5 d**	**10.0 c**	**11.5 b**		**10.1 b**			**11.4 b**	
Buffer (A)	Res	1.8 d	1.6 e	1.4 d	8.2 f	4.8 e	4.9 f		5.2 d			3.4 g	
Buffer (A)	Sup	2.1 d	2.4 e	1.7 d	5.0 h	4.3 e	2.9 i		5.2 d			3.4 g	
Buffer (A)	**Total**	**3.8 b**	**3.9 d**	**3.1 c**	**13.3 d**	**9.2 c**	**7.8 d**		**8.8 c**			**8.7 d**	
Pep and Amyl	Res	1.8 d	2.3 e	1.6 d	8.3 f	5.2 e	8.7 c		5.6 d	10.7 c		10.1 c	10.3 b
Pep and Amyl	Sup	5.7 c	7.1 b	5.1 b	7.1 g	9.2 c	6.9 e		10.1 b	9.8 d		8.2 e	7.8 c
Pep and Amyl	**Total**	**7.5 d**	**9.4 a**	**6.8 a**	**15.4 c**	**14.4 a**	**15.6 a**		**15.7 a**	**20.5 a**		**18.3 a**	**18.2 a**
Buffer (A+P)	Res	1.8 d	1.6 e	1.6 d	9.1 e	4.9 e	4.3 g		4.1 e	5.8 e		5.6 f	5.3 d
Buffer (A+P)	Sup	2.0 d	2.1 e	1.8 d	4.2 h	4.6 e	2.8 i		4.9 d	3.4 f		3.2 g	5.1 d
Buffer (A+P)	**Total**	**3.8 b**	**3.6 d**	**3.3 c**	**13.4 d**	**9.5 c**	**7.1 e**		**9.1 b**	**11.4 c**		**8.8 d**	**10.4 b**

Values within the same column with different letters are significantly different, $p<0.05$. Phenols expressed as mg catechin equivalents/g (mg CE/g), dry weight basis (Folin-Ciocalteu method)

Samples incubated in respective buffers (enzyme controls), (Buffer P)- pepsin buffer, pH 1.5 (KCl-HCl buffer) and Buffer (A), amylase buffer pH 6.9 (Tris Maleate buffer); Buffer (A+P), samples incubated in pepsin buffer, and then amylase buffer.

Abbreviations: Extrud- extrudates; Porr – porridge; Untrt-Untreated; Res-Residue; Sup-supernatant,

Table B-2
Effect of pepsin, amylase and pepsin followed by amylase treatment on antioxidant activitya of Sorghum grain, extrudates and porridges

		Macia			NS 5511			Framida			Early Sumac		
Treatment	Sample	Grain	Extrud	Porr	Grain	Extrud	Porr	Grain	Extrud	Porr	Grain	Extrud	Porr
Control	Untrtd	22±1	4±0.3	1±0	384±15	58±3	70±2	427±25	53±4	118±2	262±9	145±47	145±16
Pepsin	Res	8±2	1±0	8±2	94±6	31±6	46±2		30±7			64±16	
Pepsin	Sup	223±14	226±21	96±12	231±10	212±32	197±34		235±25			190±18	
Pepsin	**Total**	**231±12**	**227±22**	**105±13**	**325±15**	**244±26**	**244±32**		**264±21**			**254±18**	
Buffer (P)	Res	0	0	2±0	103±2	40±4	46±5		28±6			90±7	
Buffer (P)	Sup	7±1	6±2	0	62±3	50±1	30±1		68±7			32±2	
Buffer (P)	Total	7±1	6±2	8±9	166±3	91±4	76±5		95±2			122±6	
Amylase	Res	1±1	1±1	2±1	98±12	64±2	102±10		56±2			119±3	
Amylase	Sup	8±3	16±2	3±0	21±2	48±4	5±1		54±1			24±3	
Amylase	**Total**	**9±3**	**17±3**	**5±1**	**125±13**	**117±5**	**111±9**		**115±3**			**148±4**	
Buffer (A)	Res	0	0	0.1±0	100±8	54±4	45±2		41±3			120±3	
Buffer (A)	Sup	0	6±1	0	29±3	39±6	1±1		51±9			27±3	
Buffer (A)	Total	0	6±1	0	134±10	98±9	51±2		97±12			152±4	
Pep and Amyl	Res	6±5	4±2	0	83±4	58±2	127±9		44±1	143±9		94±17	108±13
Pep and Amyl	Sup	101±3	128±19	79±1	97±2	216±13	181±10		172±11	244±15		219±19	162±15
Pep and Amyl	**Total**	**118±2**	**146±20**	**93±1**	**180±5**	**274±12**	**308±18**		**216±12**	**387±22**		**313±34**	**270±28**
Buffer	Res	0	0	0	107±2	48±1	47±4		40±0	68±2		60±2	63±3
Buffer (P+A)	Sup	0	0	7±0	1±1	24±1	0		32±3	41±1		2±2	36±2
Buffer (P+A)	Total	0	0	7±0	108±2	71±1	47±4		72±3	109±1		62±3	98±5

Table B-2 continued

[a]Antioxidant activity (ABTS method) expressed as μmol Trolox Equivalents per g (μmol TE/g), dry basis (Awika et al 2003a)
[b]Extrudates and freeze dried porridges incubated in enzyme buffer suspensions, and residue and supernatant separated by centrifuging at 3 500 rpm.
[c]Samples incubated in respective buffers (enzyme controls), Buffer (P)- pH 1.5 (KCl-HCl buffer) for pepsin and Buffer (A), pH 6.9 (Tris Maleate buffer) for amylase; Buffer (P+A)- pepsin, and then amylase buffer; Res-residue; Sup-supernatant

Table B-3
Effect of pepsin, amylase and combined pepsin and amylase treatment on tannin content of residues remaining after enzyme hydrolysis of extrudates and porridges

Sample	NS 5511[a]		Framida[a]		Early Sumac[a]	
	Tannin content (mg CE/g)	% Recovered tannins	Tannin content (mg CE/g)	% Recovered tannins	Tannin content (mg CE/g)	% Recovered tannins
Grain	49.0±1.9	100	47.8±5.6	100	19.6±1.9	100
Extrudate	1.9±0.8	3.7±1.6	0.4±0.2	1.0±0.5	8.6±0.5	44.3±3.8
Extrudate-Pepsin	**0.5±0.1**	**1.1±0.3**	**ND**	**ND**	**0.2±0.2**	**0.7±0.8**
Extrudate- Buffer (P) [b]	2.3±0.2	5.4±1.1	1.1±0.2	2.4±0.4	4.7±0.9	24.4±6.9
Extrudate-Amylase	**5.3±0.3**	**10.7±0.8**	**2.7±0.6**	**5.9±1.5**	**10.4±1.6**	**54.3±13.8**
Extrudate-Buffer (A) [b]	2.3±0.6	4.7±0.9	0.3±0.6	0.8±1.4	2.8±0.2	14.4±2.2
Extrudate-Pep+Amyl	**3.2±0.3**	**6.8±0.9**	**1.1±0.3**	**2.3±0.3**	**10.7±0.6**	**60.4±4.1**
Extrudate-Buffer (P+A)[b]	0.3±0	0.4±0.3	0.01±0	0.02±0.03	0.5±0.4	2.4±2.1
Porridges	6.6±0.3	13.4±0.7	13.7±1.3	28.9±4.9	4.5±0.1	23.0±2.7
Porridges-Pep+Amyl	**12.3±0.6**	**28.5±2.0**	**14.7±2.4**	**35.1±3.1**	**10.5±1.5**	60.0±9.3
Porridges-Buffer (P+A)	2.2±0.3	3.5±1.0	3.3±0.4	6.3±0.4	1.6±0.3	7.5±1.8

Data are expressed as mean ± standard deviation of triplicate samples.
Tannins determined using the vanillin-HCl method and expressed as mg catechin equivalents (mg CE/g). All expressed on dry basis.
[a]NS 5511 and Framida extrudates were produced using a twin-screw extruder, while Early Sumac extrudates were produced on a single-screw extruder.
[b]Buffer (A)-Amylase buffer, Tris Maleate buffer, pH 6.9; Buffer (P)- KCl-HCl buffer, pH 1.5; Buffer (A+P), incubation in pH 1.5, followed by pH 6.9 buffer.
ND – Not detected.

Table B-4
Effect of enzyme treatment on phenols and antioxidant activity in bread containing white or tannin sorghum bran

Treatment	Sample	Phenols (mg CE/g)		Antioxidant activity µmol TE/g)	
		Bread containing white bran	Bread containing tannin bran	Bread containing white bran	Bread containing tannin bran
Control	Ingredients	2.2 c	6.4 b	5 c	66 c
Control	Ingredients	2.5 c	3.1 c	2 c	37 d
Pep and amyl	Residue	1.6 c	3.4 c	0 c	23 b
Pep and amyl	Supernatant	6.7 a	8.5 b	146 a	250 a
Pep and amyl	**Total**	**8.3 a**	**11.8 a**	**159 a**	**287 a**
Buffer (P+A)	Residue	1.8 c	2.2 d	0 c	9 e
Buffer (P+A)	Supernatant	2.0 c	2.3 d	0 c	8 e
Buffer (P+A)	Total	3.7 b	4.5 c	0 c	30 d
Pepsin	Residue	1.6 c	-	1 c	-
Pepsin	Supernatant	4.4 b	8.7±0.6	125 a	244±18
Pepsin	**Total**	**6.0 a**	**-**	**125 a**	**-**
Buffer (P)	Residue	1.7 c	-	0 c	-
Buffer (P)	Supernatant	1.8 c	1.7±0.1	6 c	15±1
Buffer (P)	Total	3.6 b	-	6 c	-
Amylase	Residue	2.9 c	-	6 c	-
Amylase	Supernatant	2.7 c	2.8	27 b	35
Amylase	**Total**	**5.6 a**	**-**	**33 b**	**-**
Buffer (A)	Residue	1.7 c	-	2 c	-
Buffer (A)	Supernatant	2.0 c	1.9	4 c	5
Buffer (A)	Total	3.7 b	-	6 c	-

Values within the same column with different letters is significantly different, $p<0.05$.
[a]Phenols expressed as mg catechin equivalents/g (mg CE/g), dry weight basis (Folin-Ciocalteu method).
[b]Antioxidant activity expressed as µmol Trolox Equivalents per g (µmol TE/g), dry basis (Awika et al 2003a)

Table B-4 continued

[c]Ingredients: Flour containing 12 % white or tannin sorghum bran.
[d]Untreated, freeze dried bread was incubated in buffers containing the enzymes, and residues and supernatants were separated by centrifuging.
[e]Freeze dried bread treated with pepsin, amylase or pepsin, follwed by amylase (Pep and Amyl)
[f]Buffers A, P and P+A- Enzyme control for amylase, pepsin, and pepsin followed by amylase treatment
- No data value

VITA

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